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# SOIL ACTINOMYCETES APPLIED TO BANANA PLANTS IN THE FIELD[1]

CLIFFORD H. MEREDITH

(Accepted for publication July 27, 1946)

In November, 1942, the writer (1) observed that an actinomycete, a *Streptomyces*, isolated from a compost heap in a banana area near Port Maria, Jamaica, British West Indies, was antagonistic to *Fusarium oxysporum* Schl. var. *cubense* (E.F.Sm.) Wr. and Rg., the Panama disease fungus. A survey was then made to determine the prevalence of organisms antagonistic to *F. oxysporum cubense* in banana soils in Jamaica (2). One hundred and twenty-two organisms having such antagonism in culture were isolated: 66 were slightly antagonistic, 39 antagonistic, and 17 very antagonistic. Five of the actinomycetes classed as very antagonistic, numbers 412, 512, 569, 976, and 1182 in table 1, were used in field experiments and are

TABLE 1.—*The antagonistic effect of various actinomycetes on the growth of Fusarium oxysporum cubense on Orange River soil-solution agar*

| Actinomycete[a] | Distance planted from Fusarium | Fusarium growth towards actinomycetes | | |
|---|---|---|---|---|
| | | 6 days | 20 days | 35 days |
| | *cm.* | *cm.* | *cm.* | *cm.* |
| 1182 | 1.3 | 0.5 | 0.8 | 0.6[b] |
| 569 | 1.3 | 0.7 | 0.7 | 0.7 |
| 341 | 1.3 | 0.9 | 1.0 | 1.0 |
| 448 | 1.3 | 1.0 | 1.1 | 1.1 |
| 344 | 1.2 | 1.1 | 1.0 | 1.1 |
| 487 | 1.1 | 1.1 | 1.1 | 1.1 |
| 462 | 1.3 | 1.0 | 1.1 | 1.3 |
| 976 | 1.3 | 1.0 | 0.9 | 1.3 |
| 153 | 1.3 | 1.1 | 1.3 | 1.3 |
| 412 | 1.3 | 1.0 | 1.5 | 1.3 |
| 512 | 1.5 | 1.2 | 1.0 | 1.5 |
| 947 | 2.0 | 2.0 | 2.1 | 1.9 |
| 671 | 1.4 | 1.4 | 3.4[c] | 3.6 |
| 45 | 1.1 | 3.6 | 5.3[d] | ..... |
| 918 | 1.4 | 1.4 | 5.6 | ..... |
| 188 | 1.4 | 3.0 | 6.0 | ..... |
| 1 | 1.1 | 2.8 | 6.3 | ..... |
| None | Check | 3.1 | 5.5 | ..... |
| None | Check | 2.9 | 5.4 | ..... |
| None | Check | 2.9 | 5.8 | ..... |

[a] Isolated from fields outside the Orange River area.
[b] Shrinkage indicates lysis.
[c] Where the growth is greater than the distance apart the Fusarium crossed over the line of the actinomycetes.
[d] The Fusarium had grown to the bottom of the test tube.

referred to in this paper as general actinomycetes. The antagonism of some of these isolates was substantiated by Thaysen and Butlin (4) who obtained cultures of these actinomycetes and of *F. oxysporum cubense* from the National Type Culture Collection in England.

[1] Acknowledgment is due the Jamaica Banana Producers' Association and the planters in the Parish of St. James for support in this research.

In order to test these general actinomycetes under field conditions plans were made with the Director of Agriculture for Jamaica for an extensive field experiment at the Orange River Experiment Station of the Department of Agriculture in one of the chief banana growing areas in the island. The site selected for the experiment was known to be heavily infested with *Fusarium oxysporum cubense,* as banana plants with Panama disease had been removed only a few months previously (3).

## MATERIALS AND METHODS

The general actinomycetes were grown in test tubes with *Fusarium oxysporum cubense* on Orange River soil-solution agar at 1.0 to 2.0 cm. apart. None of them exhibited as much antagonism on this medium as on agar made from their own soils, although one gave almost equal results. These actinomycetes were increased for use in the field.

Samples of Orange River soil were examined for antagonistic actinomycetes. Of those found, 1 was antagonistic and 7 were slightly antagonistic. The 1 antagonistic and 3 of the slightly antagonistic culture strains were increased, for field tests, by growing them on guinea grass and soil in liter flasks. The contents of the flasks were mixed together, thinned with water, and sprinkled over the soil and suckers before the suckers were planted. Suckers were obtained from the Orange River property.

There were five different plot treatments with 15 stools in each bed and 7 replications. The treatments were as follows:

Treatment No. 1. Check. This was the customary clean culture method of banana growing in the area.

Treatment No. 2. Mulch. Green guinea grass was cut from contour strips and placed around the stools.

Treatment No. 3. Suckers and soil were inoculated at the time of planting with a mixture of the 5 general actinomycetes.

Treatment No. 4. This was the same as treatment No. 3 with mulch added.

Treatment No. 5. Suckers and soil were inoculated at the time of planting with a mixture of the four actinomycetes from Orange River soil and mulch was added.

Green guinea grass was applied three times during the year on the mulched beds.

Suckers were planted on May 23, 1944. Growth measurements consisting of height of plant, circumference of base, width of widest leaf, and number of leaves were made on August 8 and October 10, 1944, and on May 9, 1945. Record of Panama disease occurrence was also kept.

## RESULTS

### *General Actinomycetes*

Mean growth measurements of plants in plots treated with general actinomycetes were higher than mean growth measurements of plants in check

plots in August and October readings. The difference in height in October was statistically significant.

Plants in plots treated with general actinomycetes gave higher mean growth measurements in August and October than plants in plots treated with general actinomycetes and mulch. Three of these differences were significant in August and two in October (Table 2).

TABLE 2.—*Means of growth measurements for banana plants*

| Treatment | Height | Circumference | Width of leaf | Number of leaves |
|---|---|---|---|---|
| | *inches* | *inches* | *inches* | |
| *August 8, 1944* | | | | |
| Check | 34.07 | 4.96 | 9.39 | 8.64 |
| Mulch | 29.08 | 4.69 | 8.71 | 7.87 |
| Actinomycetes | 34.51 | 5.23 | 10.24 | 9.11 |
| Actinomycetes plus mulch | 30.69 | 5.05 | 9.22 | 8.10 |
| Orange River actinomycetes plus mulch | 35.51 | 5.32 | 10.63 | 8.83 |
| F | 3.16[a] | 1.2 | 3.42[a] | 4.22[b] |
| M.S.D. | 4.35 | ..... | 1.23 | 0.73 |
| *October 10, 1944* | | | | |
| Check | 57.33 | 8.13 | 14.67 | 10.06 |
| Mulch | 57.02 | 7.87 | 14.57 | 10.47 |
| Actinomycetes | 65.14 | 9.04 | 16.05 | 10.09 |
| Actinomycetes plus mulch | 59.70 | 8.29 | 15.56 | 10.62 |
| Orange River actinomycetes plus mulch | 70.84 | 9.60 | 17.18 | 10.73 |
| F | 8.45[b] | 3.07[a] | 3.2[a] | 1.0 |
| M.S.D. | 6.03 | 1.1 | 1.73 | ..... |

[a] Significant value, 2.78.
[b] Highly significant value, 4.22.

### *The Effect of Mulch*

Plants in plots treated with mulch alone were usually smaller than unmulched plants in check plots.

Plants in plots treated with general actinomycetes and mulch were usually smaller than plants in plots treated with general actinomycetes without mulch. The difference in the number of leaves in August was significant (Table 2).

### *Orange River Actinomycetes*

Banana plants in plots treated with Orange River actinomycetes on mulch gave the highest readings in three out of four measurements in August and in all measurements in October.

In August, plants treated with Orange River actinomycetes on mulch gave significantly higher measurements in height, leaf width, and number of leaves than those treated with mulch alone and those treated with general actinomycetes on mulch. Plants treated with Orange River actinomycetes also gave significantly higher measurements in leaf width than those in check plots.

In October, plants treated with Orange River actinomycetes on mulch gave significantly higher measurements in height, circumference, and width of leaf than those in check plots and in plots treated with mulch alone. They also gave significantly higher measurements in height and circumference than plants in plots treated with general actinomycetes on mulch (Table 2).

### *Panama Disease Occurrence*

During the first five months symptoms of Panama disease appeared in 26 stools (Table 3). These stools were distributed as follows: Check, 2; Mulch, 5; General actinomycetes, 12; General actinomycetes with mulch, 3; and Orange River actinomycetes with mulch, 4. Such early attacks are generally attributed to infection in the suckers before they are planted. During the last seven months 348 stools went down with Panama disease. The final distribution of diseased stools was: General actinomycetes, 53; Check, 71; General actinomycetes with mulch, 73; Mulch, 75; and Orange River actinomycetes on mulch, 76.

TABLE 3.—*Occurrence of Panama disease in the check, mulched, and actinomycete-treated beds from date of planting, May 23, 1944, to May 9, 1945*

| Treatment | Missing stools | Plants with Panama disease | | Healthy stools, May, 1945 |
|---|---|---|---|---|
| | | May to Oct., 1944 | Oct. to May, 1945 | |
| | *No.* | *No.* | *No.* | *No.* |
| Check | 3 | 2 | 71 | 29 |
| Mulch | 3 | 5 | 75 | 22 |
| Actinomycetes | 2 | 12 | 53 | 38 |
| Actinomycetes plus mulch | 3 | 3 | 73 | 26 |
| Orange River actinomycetes plus mulch | 1 | 4 | 76 | 24 |

## DISCUSSION

The general actinomycetes which had been classed as "very antagonistic" on their own soil had less effect on growth of banana plants in Orange River soil than Orange River actinomycetes, in spite of the fact that the latter had been classed as "antagonistic" and "slightly antagonistic." This fact raises the question of the essential nature of such antagonism.

Mulch, in these experiments, appeared to be detrimental to the growth of banana plants. This is in agreement with the fact that clean culture methods of banana cultivation have been found to be most satisfactory in Jamaica.

The plants in plots treated with Orange River actinomycetes and mulch gave the highest measurements the greatest number of times. The detrimental effect of mulch was apparently overcome to some extent by the presence of antagonistic actinomycetes functioning on their own soil.

The Orange River plot on which these experiments were carried out was known to be heavily infested with *Fusarium oxysporum cubense*. Some of the plots from which the "very antagonistic" general actinomycetes were taken were known to be areas in which the spread of the disease had been less rapid than was the general rule. Even though the disease was present, over a period of several years fewer plants had succumbed to it than in nearby areas. The nature of this "natural resistance" was not known. Waksman (5) states that "20 to 40 per cent of all actinomycetes are known to be capable of producing antibiotic substances." This might have some bearing on the case as the most antagonistic actinomycetes came from fields where natural resistance was high.

Although the plots treated with the general actinomycetes produced the largest number of plants free from Panama disease, the difference was not significant because of the wide variation in the number of diseased plants in the replications within the treatments.

There was also indication that the mulch increased the occurrence of Panama disease but again the difference was not significant. It must be pointed out that only one application of actinomycetes was made in these experiments at the beginning when the plants were set out.

## SUMMARY

Significant differences in growth measurements were found to exist between plants grown on plots treated with actinomycetes of varying degrees of antagonism and those grown without such treatment.

Treatment with "antagonistic" and "slightly antagonistic" actinomycetes on their own soil resulted in greater growth increases in banana plants than did the treatment with "very antagonistic" actinomycetes from other soils.

Growth measurements of plants treated with mulch did not compare favorably with those of unmulched plants.

Plots treated with "very antagonistic" actinomycetes from other soils produced fewer plants with Panama disease than did plots treated with "antagonistic" and "slightly antagonistic" actinomycetes from Orange River soil.

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# HELMINTHOSPORIUM ROT OF TOMATO FRUITS

L. P. McCOLLOCH[1] AND FLORA G. POLLACK[2]

(Accepted for publication August 8, 1946)

The demand for fresh tomatoes in the United States from November until May is greater than the domestic supply. The importation of tomatoes during this period is principally from Mexico, Cuba, and Haiti. Mexico alone shipped more than 8,000 carloads of "green-wrapped" tomatoes into the United States and Canada during the 1944–45 season (9, 10, 11). The producing areas are largely confined to three States in Mexico; Sonora and Sinaloa on the west coast with an estimated 49,000 acres planted for the 1945–46 season, and Tamaulipas on the east coast with 6,000 acres.

In the past 11 years United States plant quarantine inspectors have occasionally intercepted fruits with decayed spots that were unlike the usual tomato diseases encountered in the domestic crop. The first interception of this disease was made from a shipment of tomatoes from Mexico in April, 1934, by J. R. Wood of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Since that time 16 additional interceptions of diseased fruit from Mexico, 2 from Haiti, and 1 from British Guiana have been made by plant quarantine inspectors.

The diseased specimens were sent to John A. Stevenson of the Division of Mycology and Disease Survey, U. S. Department of Agriculture, who suggested that the disease and causal organism be studied.

## DESCRIPTION OF DISEASE

Specimens of the rot intercepted at ports of entry were characterized by circular to irregular areas on the fruit, 10–30 mm. in diameter, covered by a dense weft of dark gray to almost black mycelium which completely obscured other spot characteristics.

The fungus was isolated from the intercepted specimens and the isolates were used to inoculate mature green tomatoes, and from these the characteristics of the rot were observed.

The lesions were flattened to slightly sunken, at first circular, but becoming irregular as the decay advanced. Considerable variation was found in the surface coloring of the lesions, but in general it was distinct from other tomato rots. The tissue about the point of infection was usually dark or black. The most variable character was the extent of the cream-colored area surrounding the dark center. It was nearly always present at temperatures of from 60° to 80° F. and served as a striking diagnostic character (Fig. 1, A, B). A darker area ranging from bay to burnt sienna to mahogany red[3]

[1] Associate plant pathologist, Division of Fruit and Vegetable Crops and Diseases and [2] assistant mycologist, Division of Mycology and Disease Survey; Bureau of Plant Industry, Soils, and Agricultural Engineering; Agricultural Research Administration; United States Department of Agriculture; Beltsville, Maryland.

[3] The descriptive color terms used in this paper are those given in Robert Ridgway's Color Standards and Color Nomenclature, 1912.

surrounded the cream-colored area. Occasionally there were lesions that advanced slowly at 70° F., in which the cream-colored area never developed (Fig. 1, C). Such lesions resembled those caused by *Alternaria*. At lower temperatures, especially at 50° F., the spot was poorly defined; the fungus penetrated the tomato so slowly that at the end of a week the tissue was not completely killed, but had a bronzy appearance (Fig. 1, D). When these

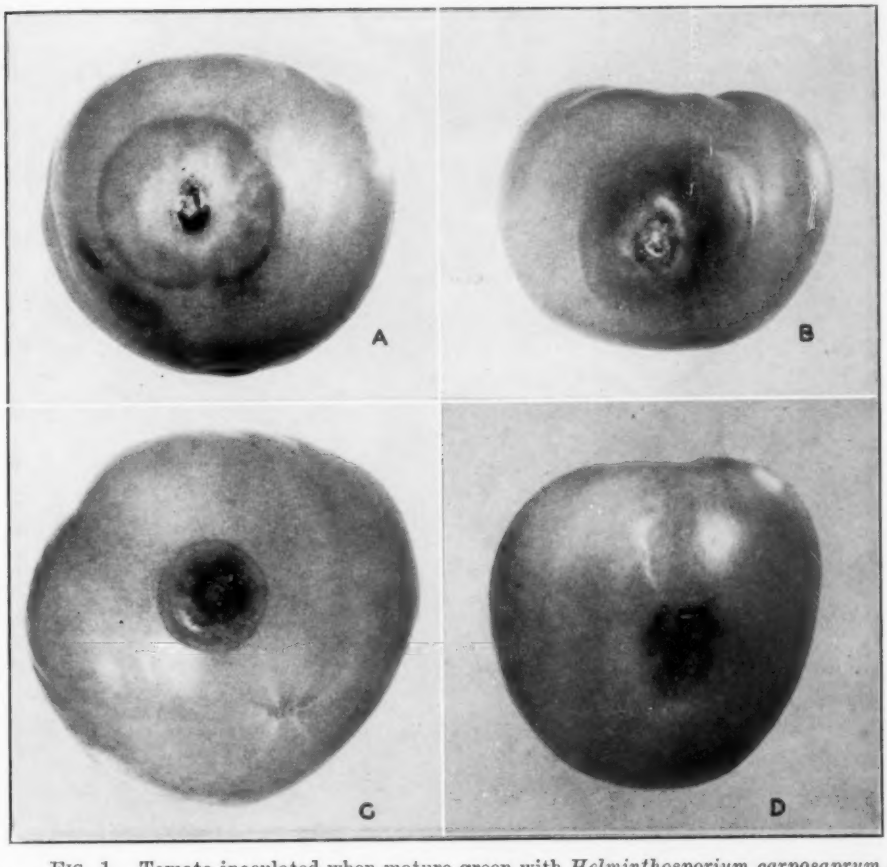


FIG. 1. Tomato inoculated when mature green with *Helminthosporium carposaprum*. A and B, typical decay at 70° and at 80° F.; C, variation also produced at 70° F., which resembled Alternaria rot. D, symptoms of infection produced at 50° F. Photographs by Lilian A. Guernsey.

fruits were transferred to higher temperatures the lesions gradually became like those developed at higher temperatures.

The rot consisted of a firm spongy mass of decayed tissue which extended deeply into the fruit but caused no offensive odor. When fruits with advanced decay were cut, dark gray mycelium was evident through the necrotic tissue.

## MORPHOLOGY OF THE FUNGUS

Sporiferous areas can usually be located on diseased tomatoes by their

darker color, which is due to the dark-colored conidiophores borne singly or in clusters (Fig. 2, B) on the skin of the tomato. Conidiophores are erect, many-septate, 140–500 × 6–10 μ, sometimes tapering to a width of 4–6 μ at the apex which is often paler. The base of the conidiophore (Fig. 2, A) con-

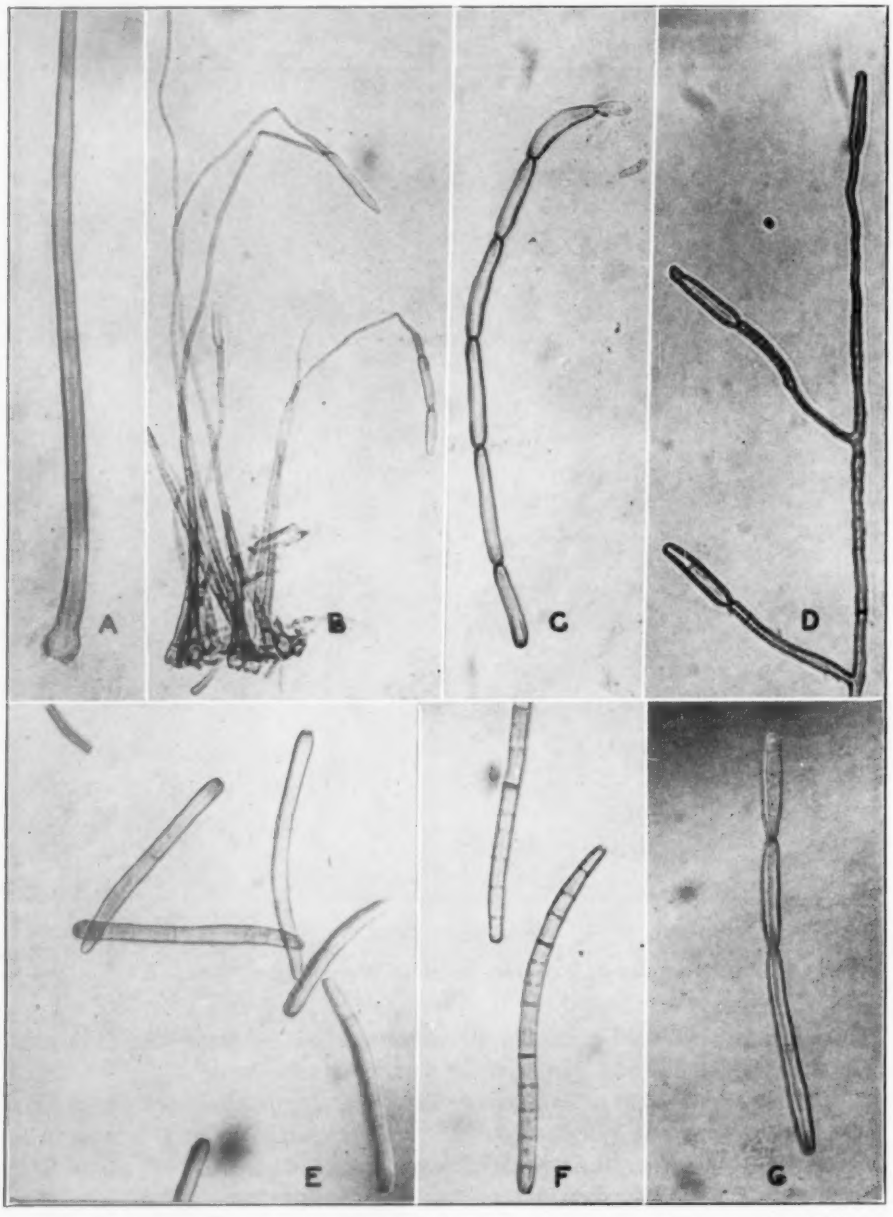


FIG. 2. *Helminthosporium carposaprum:* A, enlarged conidiophore with bulbous base; B, typical conidiophores with conidia, developed on the skin of tomato; C, conidial chain; D, type of conidiophore developed in culture; E, F, and G, variations in conidia. Photographs A and B by Marcel Foubert, C through G by Lilian A. Guernsey.

sists of a thick-walled, dark brown, bulb-like cell, 12–16 × 10–12 μ. Under conditions of high humidity conidiophores are produced as lateral or terminal branches of the hyphae (Fig. 2, D). These are usually narrower (4–6 μ) and may be very short projections from hyphae, or as long as the conidiophores arising from a fruit. Geniculate conidiophores were not observed.

Conidia are usually cylindrical, occasionally tapering, with an obtuse to rounded basal end and apex. The apex tends to become obtuse when secondary conidia are formed there. Long chains of acropetally produced conidia are frequently observed (Fig. 2, C). The conidia are sub-hyaline to dilute olivaceous, straight or curved, 1–15 septate, 28–220 × 6–12 μ, usually 120–160 × 8–10 μ (Fig. 2, E, F). Budded conidia are usually smaller, paler in color and often continuous (Fig. 2, G). Spores germinate by the production of one or two polar germ tubes which pass through the inconspicuous hila that may be formed at both basal and apical ends of spores.

## SYSTEMATIC TREATMENT

From specimens received in 1934 and 1938, John A. Stevenson recognized the causal fungus to be distinct from *Helminthosporium tomato* Ell. and Barth. (3) which is reported to occur on decayed tomatoes. In 1942 Miss Edith Cash working on two other specimens reported that conidial dimensions were similar to those of *Helminthosporium lycopersici* Maub. and Roger, but that conidiophore length was different. *H. lycopersici* was described from tomato leaves in Africa by Maublanc and Roger (5) July 10, 1936. Roldan (6) used the same binomial for a fungus which he described from leaves of *Lycopersicon* from Luzon, Philippine Islands, in the same year. The account of his fungus appears in the June, 1936, issue of the Philippine Journal of Science, which was not actually published until September 2, 1936, as noted in the table of contents of volume 60, for 1936. Maublanc and Roger, therefore have priority in the use of the name. It has not been possible to determine whether the two fungi are synonyms due to lack of specimens for comparison and to the inadequacy of the description of Maublanc and Roger.

Comparison of Roldan's description and illustrations with the *Helminthosporium* under investigation here revealed differences in size, shape, and color of the fructifications. A specimen of *Helminthosporium rhopaloides* Fres. reported on dead tomato stems has been examined and found to have shorter and darker conidia and conidiophores (7; 8, Fig. 831).

For the fungus under discussion, which does not appear to have been described, the name *Helminthosporium carposaprum* is proposed, the specific epithet being suggestive of the fruit rotting effect.

## DIAGNOSIS[4]

*Helminthosporium* **carposaprum** Pollack sp. nov.

Maculae in fructibus Lycopersici esculenti plerumque per mycelium prae-

[4] The authors wish to express their appreciation to Miss Edith K. Cash for her help in the preparation of the Latin diagnosis, and to John A. Stevenson for his suggestions.

crassum fumentem usque nigellum vestitae; conidiophora in peripheria maculae, singula vel caespitosa, fusca, recta, multi-septata, 140–500 × 6–10 μ ad apicem 4–6 μ crassum, pallidum attenuata, basi obscuriori, pachypleuro, bulboso; conidia acrogena, cylindracea, ad basim et apicem obtusa usque rotundata, interdum attenuata, recta vel curvata, subhyalina usque dilute olivacea (obscuro olivaceo-bubalina Ridgway), 1–15 septata, 28–220 × 6–12 μ frequenter 120–160 × 8–10 μ plerumque catenulata.

Lesions on tomato fruit predominantly cream-buff with a darkened area around the infection point, with the outer zone of the spot bay, burnt sienna, or mahogany red. Spot characteristics usually masked by an overgrowth of a thick weft of dark gray to almost black mycelium.

Conidiophores appearing around the edge of the spot, singly or in clusters, dark, erect, many-septate, 140–500 × 6–10 μ, sometimes tapering to a width of 4–6 μ at the relatively paler apex, with a dark, thick-walled, bulbous basal cell, 12–16 × 10–12 μ, attached to the skin of the fruit.

Conidia produced at the tips of the conidiophores only, usually in long chains, cylindrical with obtuse to rounded base and apex, sometimes tapering, straight or curved, sub-hyaline to dilute olivaceous (dark olivaceous buff according to Ridgway), 1–15 septate, 28–220 × 6–12 μ, most often 120–160 × 8–10 μ, germinating by means of one or two polar germ tubes passing through the hila.

On fruit of *Lycopersicon esculentum* grown in Mexico, Haiti, and British Guiana. Specimens are deposited in the Mycological Collections, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture (Type No. 71481 on inoculated tomatoes).

### CULTURAL RESPONSES

*Helminthosporium carposaprum* grew readily on various nutrient media such as cornmeal, string bean, prune, and potato-dextrose agar. The fungus also flourished on autoclaved green stems of tomato and of a coarse wild grass. Growth at 70° to 80° F. was rapid and the fungus sporulated within 5 to 7 days. Irregularities in the size and shape of spores and conidiophores are common in culture. Conidiophores arise only as lateral or terminal outgrowths of hyphae and are scarcely distinguishable from the mycelium. The conidia are smaller in culture than on the tomato fruit, and frequently produce secondary spores which in turn often produce still other spores. Long chains of these spores are common in culture as well as in nature. Similar tendencies toward catenulation have been reported by Drechsler (2), by Berg (1), and by Olive (4). Another characteristic in culture is the production of sporophoric processes from the apices of spores, and chains of spores interspersed with these sporophoric structures are often seen. This characteristic has also been reported by Drechsler (2) and by Berg (1).

### TEMPERATURE STUDIES

In the temperature studies of the fungus in the fruit, the temperature range was primarily confined to those encountered in commercial handling.

However, inoculated tomatoes were stored at 32°, 36°, and 40° F. to observe the possibility of infection at these lower temperatures. This wider range was also employed in the studies on nutrient media.

### ON TOMATO FRUIT

Growth measurements of the fungus lesions were made in order to obtain the rate at which decay developed at 80°, 70°, 60°, 55°, and 50° F. Mature green tomatoes used in this study were either purchased in a Washington, D. C., market or grown at the Plant Industry Station. A series of four tests were made using from 12 to 24 fruits for each temperature in each test. The fruits were washed in 50 per cent alcohol, dried, and inoculated by inserting bits of mycelium in small oblique wounds. One inoculation was made per fruit. The fruits were wrapped and stored at the above temperatures. Measurements were made after 7, 14, and 21 days had elapsed. Figure 3 shows the effect of temperature on the rate of development of the lesions.

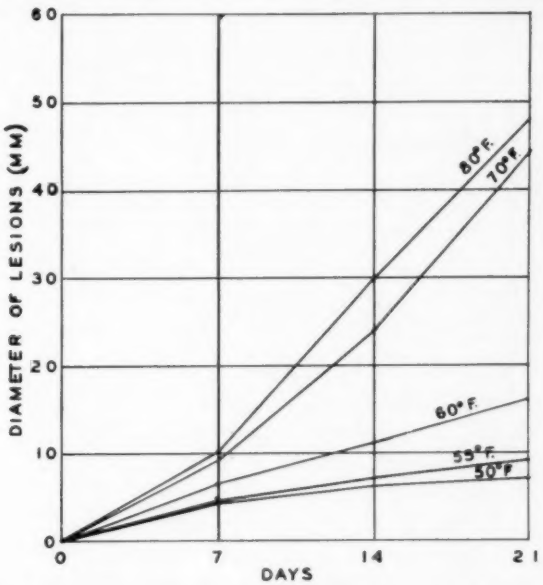


FIG. 3. Effect of temperature on rate of development of lesions on mature green tomatoes produced by *Helminthosporium carposaprum*.

Decay lesions developed slowly at all temperatures during the first week. At the end of 14 days and thereafter, there was a sharp contrast between development at the higher temperatures of 70° and 80° and that at 60° F. and below, as is shown in figure 3. The indications are that a sizable rot can develop on mature green tomatoes during shipment and ripening when temperatures are about 70° F. or above. However, the fungus was tolerant of lower temperatures. While decay at 60° F. and below was slow, it was in such a stage of development that the lesions enlarged rapidly when the fruits were removed to the ripening room at 70° F. The fruits were weakened by

subjection to 32°, 36°, and 40° F.; no infection was evident at 32° or 36° during 30 days but there was slight infection at 40° F. after 10 to 14 days.

ON AGAR PLATES

Measurements to obtain the rate of growth of the fungus in culture were made at 80°, 70°, 60°, 55°, 50°, 40°, 36°, and 32° F. Five to 10 Petri plates with 20 cc. of Czapek's agar were used at each temperature and the test was repeated. The inoculum, consisting of a small amount of mycelium, was planted in the center of the agar and the plates were stored immediately. Measurements of the diameter of the colonies were made after 7, 14, and 21 days.

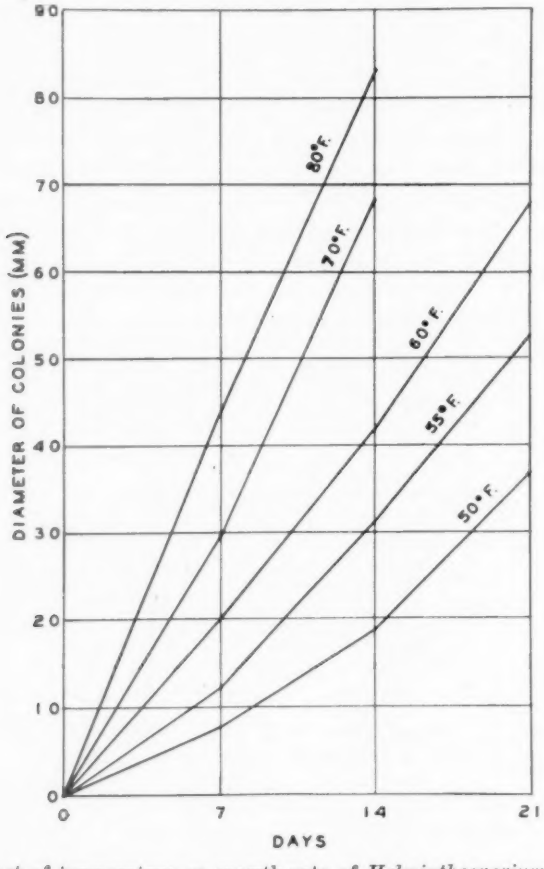


FIG. 4. Effect of temperature on growth rate of *Helminthosporium carposaprum* on Thaxter's agar in Petri plates.

Figure 4 shows that the *Helminthosporium* growth rate was approximately twice as rapid at 80° as at 60° F., fully twice as rapid at 70° as at 55° F. and about twice as rapid at 60° as at 50° F. No measurable growth occurred at 40°, 36°, or 32° F. during 21 days. However, when examined with a hand lens, the inoculum in those plates stored at 40° was found to be

covered with new growth, while at 36° and 32° F. it seemed dormant. Colonies developed when these plates were transferred to room temperature.

A comparison of the diameter of the fungus colonies on agar with the diameter of the lesions on the fruit at the end of 3 weeks showed that the colonies on agar increased from 2 to 5 times as much as the lesions on the fruit at corresponding temperatures.

### INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

Spores were placed in sterile water and in diluted prune juice and stored at 80°, 70°, 60°, 55°, 50°, 40°, 36°, and 32° F. Within 24 hours germination had started at 50° F. and above. By the end of 72 hours, some spores were germinating at 40° F. and after 9 days about 35 per cent of these spores had germinated. Spores held at 36° and 32° were found to be dormant even after 22 days.

### EFFECT OF FREEZING

Vigorous cultures of the *Helminthosporium* on Thaxter's agar slants were placed at 16° F. to determine the effect of such a low temperature on the survival of the fungus. Transfers from withdrawals after 6, 7, 9, 14, 21, and 51 days gave viable cultures, but cultures exposed for 63 days were dead.

### INOCULATION EXPERIMENTS

#### *Tomato Plants*

As most species of *Helminthosporium* are associated with leaf-spotting diseases it seemed of interest to test the pathogenicity of the tomato fruit-rotting fungus on tomato plants. Potted plants that had started to bloom were used for these tests. The leaves were punctured with a needle, making one or two groups of holes in each leaflet. The inoculating was done by atomizing an aqueous spore suspension on the plants. Inoculated and non-inoculated plants were then placed in a humid chamber designed to keep the air automatically saturated with water.

Extensive spore germination was observed in 24 hours in the unused portion of the inoculum which was left in the humid chamber with the plants. The margin of the punctures in the leaves became blackened after 24 hours, but there was never any manifestation of disease.

The experiment was repeated, and for part of the material to be inoculated plants were selected that were pot bound and physiologically weakened, as shown by a general yellowing of the foliage. In the second test the vigorous plants (Fig. 5, B) again failed to develop the disease but necrotic lesions developed about the punctures of the devitalized plants (Fig. 5, A), from which the *Helminthosporium* was recovered. These experiments indicate that the fungus did not cause a leaf spot of tomato when the plants were in a reasonably good state of vigor. In addition to the leaf inoculations, mats of mycelium were inserted into the plant stems, but failed to become established or cause lesions.

*Tomato Fruits*

Mature green tomatoes, when inoculated by inserting a mat of mycelium, developed a sizable rot by the time the fruit was ripe, as is shown in the discussion on temperature studies.

To test a smaller amount of inoculum, mature green tomatoes were punctured with a sterile needle and dipped in an aqueous spore suspension. Test lots were stored at 40°, 50°, 55°, 60°, and 70° F. Infection was erratic and slow. Lesions 1 to 2 mm. in diameter were evident on some fruits stored at 60° F. for 9 days.

FIG. 5. A, Leaf from devitalized tomato plant showing Helminthosporium lesions forming around points of inoculation. B, Leaf from vigorous plant failed to develop the disease. Photograph by Lilian A. Guernsey.

*Other Plant Products*

To study the behavior of this fungus further, fruits from certain other plants were inoculated by inserting a mat of mycelium under the skin, and storing them in moist chambers at 60° F.

Slow decay developed on York Imperial apples (*Malus sylvestris* Mill.), scallop squashes (*Curcurbita pepo* Mill.); moderate decay developed on eggplant fruit (*Solanum melongena* L.) and bell peppers (*Capsicum frutescens* var. *grossum* Bailey). The fungus was unable to cause infection in orange (*Citrus sinensis* L. Osbeck) or lemon (*Citrus limon* (L.) Burm. f.) fruits.

The decay lesions on the apple fruits were firm and remained small, about 10 mm. in diameter, even though the fruits were held until they broke down

from over-ripeness. Round, firm lesions from 6 to 8 mm. in diameter developed on scallop squashes in 9 days. The firm, round sunken lesions developed slowly on eggplant at first, measuring 10 to 15 mm. in diameter in 9 days, but the decay continued to spread and eventually consumed a large part of the fruit. Decay developed more rapidly on bell peppers with lesions of irregular patterns.

## DISCUSSION

The studies on *Helminthosporium carposaprum* indicate that it is a weak parasite both in the field and on mature green tomato fruits during transportation and subsequent ripening. The fungus failed to produce leaf spots or stem lesions on vigorous tomato plants in spite of wounding and favorable infection conditions. Lesions developed, however, around puncture wounds when plants in a low state of vigor were inoculated. The fruits appear to be more susceptible than other parts of the plant, but a sizable amount of inoculum is necessary to produce a decay that will affect the marketability. It seems probable that in the field the fungus grows on weakened plant tissue and debris and that this material then becomes a source of infection for tomato fruits.

From the limited reports at hand, *Helminthosporium carposaprum* does not appear to cause a serious disease. From observations of infection at 40° and 50° F. it seems probable that infection is encouraged by low temperatures and light frosts unfavorable to the fruit in the field, such as are encountered in Mexico during the growing and harvesting season (9, 10, 11). It is believed that the interception of tomatoes with sizable decay at ports of entry is the result of careless sorting and grading rather than the rapid development of the fungus in transit.

From inspectors' records for the past eleven years, there seems to have been no extensive increase in this disease, and no record of its presence in the United States. In view of this, and from the results of these studies, it seems unlikely that this fruit rot will reach serious proportions in the United States.

## SUMMARY

The study of an undescribed rot of tomato fruits intercepted in imports from certain tropical or subtropical regions is reported. The cause of the disease, demonstrated by pure culture inoculations, is an undescribed species of *Helminthosporium*. This is now described under the name *Helminthosporium carposaprum*.

Inoculation of tomato stems, foliage, and mature green fruit indicated that the fungus is a weak parasite.

The fungus caused slow to moderate decay when inoculated into the harvested fruits of eggplant, bell pepper, scallop squash, and apple, but not of orange or lemon.

Temperature studies of the fungus on tomato fruits and on artificial media indicated tolerance to a wide range of temperature. Of the tempera-

tures tried, the best growth was obtained at the higher temperatures, 70° and 80° F. There was slight development of the fungus at as low as 40° F. and the fungus remained viable after 51 days at 16° F.

The *Helminthosporium* fruit rot of tomato does not appear to have increased significantly during the past 11 years. Although tomatoes from those areas in which the disease is found have been imported during this time there is no record of occurrence of the disease in the United States.

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# THE EFFECT OF FUNGICIDAL SEED TREATMENTS ON GUAYULE SEEDLING EMERGENCE

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## INTRODUCTION

In 1942 and 1943 the Guayule Emergency Rubber Project, under the direction of the U. S. Forest Service, established several large guayule (*Parthenium argentatum* Gray) nurseries in California for the production of planting stock. Pre-emergence losses in these nurseries and especially those located at Salinas where most of the field planting stock was produced, ranged from moderate to heavy. The average seedling emergence varied from 40 to 50 per cent of the germinable seed sown and seldom exceeded 75 per cent. Because of poor and unsatisfactory emergence in 1943 approximately one-fourth of the nursery beds had to be resown in two of the Salinas nurseries. Damping-off fungi, *Pythium* and *Rhizoctonia,* have been reported (8) as the principal cause of pre-emergence losses. Post-emergence damping-off did occur but the losses were comparatively low in 1942 and 1943 when the guayule nurseries were being operated on a large-production scale.

As a part of the general research program to facilitate the guayule rubber emergency production program, investigational work was undertaken to determine the cause or causes of poor seedling emergence and to develop control measures. The results of greenhouse, nursery, and field tests are herein reported.

## DAMPING-OFF FUNGI

Various fungi have been isolated by the writer and others (4, 5, 7) from young guayule seedlings affected with damping-off and seedling root rot. Of these, *Pythium ultimum* Trow was predominant. However, various Fusaria were isolated frequently and a *Rhizoctonia sp.* was isolated occasionally. Several other fungi, considered to be non-pathogenic and commonly obtained in culture from plants affected with damping-off, were also obtained.

Pathogenicity tests were made in the greenhouse with many of the isolates obtained from diseased seedlings. All of the isolates of *Pythium ultimum* and *Rhizoctonia* tested were found to be virulent pre-emergence and seedling root-rot pathogens. The results were inconclusive as to the pathogenicity of the several Fusaria isolated. *Phytophthora drechsleri* Tucker, which was isolated from root-rot lesions on older plants (7), was found to attack and kill emerging guayule seedlings but was not isolated from damped-off nursery seedlings.

Of the many fungi associated with damping-off in the nursery and field

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sowings in California, *Pythium ultimum* is considered the most important. However, the sum total of the losses caused by the other fungi may be much greater than now suspected.

SEED

Both threshed and unthreshed guayule seed have been used in nursery and direct field sowings. However, most of the seed sown for production purposes has been unthreshed. Threshed seed has been used in both nursery and direct field sowings in large scale tests and was found to be equal or superior to the unthreshed seed. Both sorts of seed were used in testing various fungicidal seed treatments. The unthreshed seed (2, 6) consists of an achene with attached floral parts, and in threshed seed the accessory floral parts have been mechanically removed from the achene.

The percentage of filled or potentially viable seed varied greatly between different lots and was usually lower in the unthreshed than in the threshed seed. In the threshing and cleaning process many of the unfilled seed were removed. The percentage of filled seed usually ranged from 30 to 40 in the unthreshed lots, and from 60 to 70 in the threshed lots. These wide variations in filled seed, as well as in the percentage of germinable seed,[2] account for many of the discrepancies in seedling emergence between tests.

It has been a standard practice to pretreat unthreshed seed with sodium hypochlorite. Essentially the treatment (1) consists of soaking the seed in water for 18 to 20 hours and then treating the seed for 2 hours with a solution of sodium hypochlorite containing 1.0 to 1.5 per cent available chlorine, after which the seed is thoroughly washed, dried, and stored. The purpose of the hypochlorite treatment is to increase the germinable percentage of recently harvested guayule seed. Benedict and Robinson (3) found that the hypochlorite treatment accomplished this primarily by increasing the permeability of the seed coat which surrounds the embryo. While the hypochlorite treatment (pretreatment) is not applied for its fungicidal action, it effectively eliminates surface seed-borne organisms. Since both hypochlorite-treated and untreated seed may be treated with seed protectants (fungicides), the hypochlorite treatment is referred to as a pretreatment. Threshed seed may also be pretreated with sodium hypochlorite, although its practical value is questionable. Benedict and Robinson (3) have shown that either threshing or aging in the open, or both, will improve seed germination. They conclude "that in so far as germination is concerned the very expensive (sodium hypochlorite) seed treatment now given guayule seed can be eliminated by using achenes alone that have been stored in the open for six months or by producing seed in (hot) climates such as that at Indio, California, and holding it for a year before planting."

FUNGICIDES

A number of seed protectants were tested for pre-emergence damping-off control on both threshed and unthreshed guayule seed which had or had not

[2] By germinable seed is meant those seed which will germinate readily, in 10 to 14 days, under usual seed laboratory or greenhouse conditions.

been pretreated with sodium hypochlorite. The fungicides tested for pre-emergence damping-off control were Arasan (50 per cent tetramethyl thiuramdisulfide), Fermate (70 per cent ferric dimethyl dithiocarbamate), Cuprocide (98 per cent red cuprous oxide), Spergon (98 per cent tetrachloro-para-benzoquinone), Spergonex (*o*-benzoquinone dionium peroxide), Semesan (30 per cent hydroxymercurichlorophenol), U.S.R. No. 604 (2,3-dichloro-1,4-naphthoquinone), and Mersolite-19 (phenyl-mercuric-salicylate).

Of the various fungicides used, all have been reported on as seed protectants by various workers at one time or another on crops other than guayule with the exception of Mersolite-19 (9). Since reports on their efficacy may be found readily in the literature, no specific citations are given.

The fungicides were applied as a dust to dry seed. The most commonly used fungicidal dosage was 1 per cent by weight. The seed to be treated or dusted and the desired amount of fungicide were placed in an airtight container, either a small jar or a rotary seed treater depending on the amount of seed to be treated, and thoroughly mixed by shaking or rotating the container for approximately 5 minutes. When 300 seeds or less were to be sown in a single flat or row the seeds were counted and placed in a coin envelope after the fungicide was applied. The usual practice was to sow within 3 or 4 days after treatment. However, delays of 10 to 14 days or longer did not reduce seedling emergence.

### GREENHOUSE TESTS

Unless otherwise indicated, 200 seeds were sown in each replication. The seeds were sown on soil in flats and covered with about ¼ inch of pasteurized sand. The sand was kept moist by frequent watering in order to secure maximum emergence. Seedling emergence was counted daily. The effect of the various seed treatments is based upon the total number of seedlings that emerged by the 15th day.

In most of the emergence tests in the greenhouse both pasteurized and fungus-infested soil were used. Damping-off fungi were eliminated from raw soil by means of a continuous soil pasteurizer (10). In the 6 minutes required for passage through the pasteurizer the soil was heated to 200° to 210° F., a temperature considerably higher than the 150° F. used by Tavernetti (10) to control damping-off caused by species of *Pythium* and *Rhizoctonia*. Fungus-infested soil was obtained by infesting pasteurized soil with various isolates of *Pythium* and *Rhizoctonia*, which had been isolated from nursery damped-off guayule seedlings.

#### *Effective Fungicidal Dosage*

Preliminary tests were made to determine, within limits, the fungicidal dosage required to give adequate pre-emergence damping-off control in fungus-infested soil. In general, the most effective dosage for Arasan, Semesan, Spergon, Spergonex, and Cuprocide was 1 per cent when used on either threshed seed or on unthreshed seed which had been pretreated with

TABLE 1.—*Effect of 7 different fungicides on guayule seedling emergence*

| Sort of seed | Soil treatment | Fungicide[a] and seedling emergence, 15 days after sowing | | | | | | | | Difference required for significance |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mersolite-19 | No. 604 | Arasan | Fermate | Spergon | Semesan | Spergonex | Check | |
| | | *Mean* | *Mean* | *Mean* | *Mean* | *Mean* | *Mean* | *Mean* | *Mean* | |
| Threshed | Pasteurized[b] | 156.6 | 149.2 | 140.4 | 136.8 | 140.6 | 141.6 | 98.2 | 112.8 | 12.70 |
| | Infested | 156.7 | 134.7 | 141.4 | 76.0 | 22.0 | 39.1 | 5.2 | 11.5 | 10.73 |
| | Nursery | 139.3 | 134.6 | 119.3 | 37.0 | 15.6 | 16.2 | 4.4 | 3.8 | 13.92 |
| Unthreshed | Pasteurized | 61.1 | 52.7 | 60.3 | 56.1 | 54.6 | 57.7 | 50.8 | 49.8 | 6.93 |
| | Infested | 56.9 | 52.3 | 54.4 | 21.2 | 10.3 | 39.8 | 3.0 | 2.6 | 6.00 |
| | Nursery | 35.5 | 45.4 | 50.8 | 14.7 | 7.0 | 21.4 | 1.5 | 1.3 | 3.79 |
| Unthreshed, pretreated with hypochlorite | Pasteurized | 37.7 | 91.7 | 113.7 | 107.9 | 105.9 | 77.8 | 99.1 | 94.0 | 12.46 |
| | Infested | 63.5 | 97.9 | 103.0 | 104.6 | 101.3 | 104.0 | 104.8 | 56.1 | 12.49 |
| | Nursery | 54.6 | 101.4 | 114.5 | 103.2 | 107.0 | 90.7 | 83.5 | 40.4 | 7.54 |

[a] The seed treatment dosage for Mersolite-19 was 0.25 per cent; for No. 604 it was 0.75 per cent, and it was 1 per cent in the other treatments.
[b] Only 5 replications of 200 seeds for each seed treatment; 10 replications in all other tests.

sodium hypochlorite. No apparent injury was caused by any of these 5 fungicides, at dosages of 1 per cent or less, to the germinating seed and emerging seedlings in either pasteurized soil or soil infested with *Pythium* and *Rhizoctonia*.

Seedling emergence was better in both pasteurized and fungus-infested soil when the seeds were treated with a 0.25 per cent dosage of No. 604 than with 0.5 or 1 per cent dosages of the same protectant. In the case of the pasteurized soil this difference was large enough to be significant indicating the possibility of chemical injury from dosages of 0.5 per cent or higher. However, this indication of injury was partially offset by the results from the fungus-infested soil where the difference in emergence between the 0.25 and 0.5 per cent dosages was not significant. On unthreshed hypochlorite-treated seed the emergence resulting from the 3 different dosages did not differ greatly.

### *Comparative Seed Treatment Tests*

Three greenhouse tests (Table 1) were made to compare the effectiveness of 7 different fungicides in the control of pre-emergence damping-off when used on either threshed or unthreshed seed, or on unthreshed sodium hypochlorite-treated seed. The fungicidal dosages used were 0.25 per cent for Mersolite-19, 0.75 per cent for No. 604, and 1 per cent for Arasan, Spergon, Semesan, and Spergonex. There were 10 replications of each treatment in pasteurized soil, in soil which had been infested with cultures of *Pythium* and *Rhizoctonia*, and in raw or nontreated soil from the Alisal Nursery. Damping-off fungi were prevalent in the nursery soil. On the basis of isolations from affected seedlings the most prevalent was *Pythium ultimum*.

In nursery and inoculated soil infested with *Pythium* and *Rhizoctonia* the increase in emergence from seed treated with Arasan, No. 604, and Mersolite-19 was significantly greater than for any of the other treatments on both threshed (Fig. 1) and unthreshed seed which had not been pretreated with sodium hypochlorite (Table 1). On unthreshed seed which had been pretreated with sodium hypochlorite all of the fungicidal treatments increased emergence about equally except Mersolite-19 which gave little or no increase in emergence over the checks. With the exception of Mersolite-19 on hypochlorite-treated seed, there was little or no indication that the fungicides at the dosages used in these 3 tests caused any chemical injury. In all probability the 0.25 per cent dosage of Mersolite-19 was excessive when dusted on hypochlorite-treated seed and the chemical injury which resulted tended to offset its protective value.

The effects of the different fungicides on the rate of emergence when dusted on both threshed seed and hypochlorite-treated unthreshed seed sown in fungus-infested soil are given in figure 1. Seedling emergence was well under way by the 4th day after sowing for most of the treatments and continued at a rapid rate until about the 7th or 8th day when it began to level off.

Seedling emergence tests were made to determine if the standard sodium

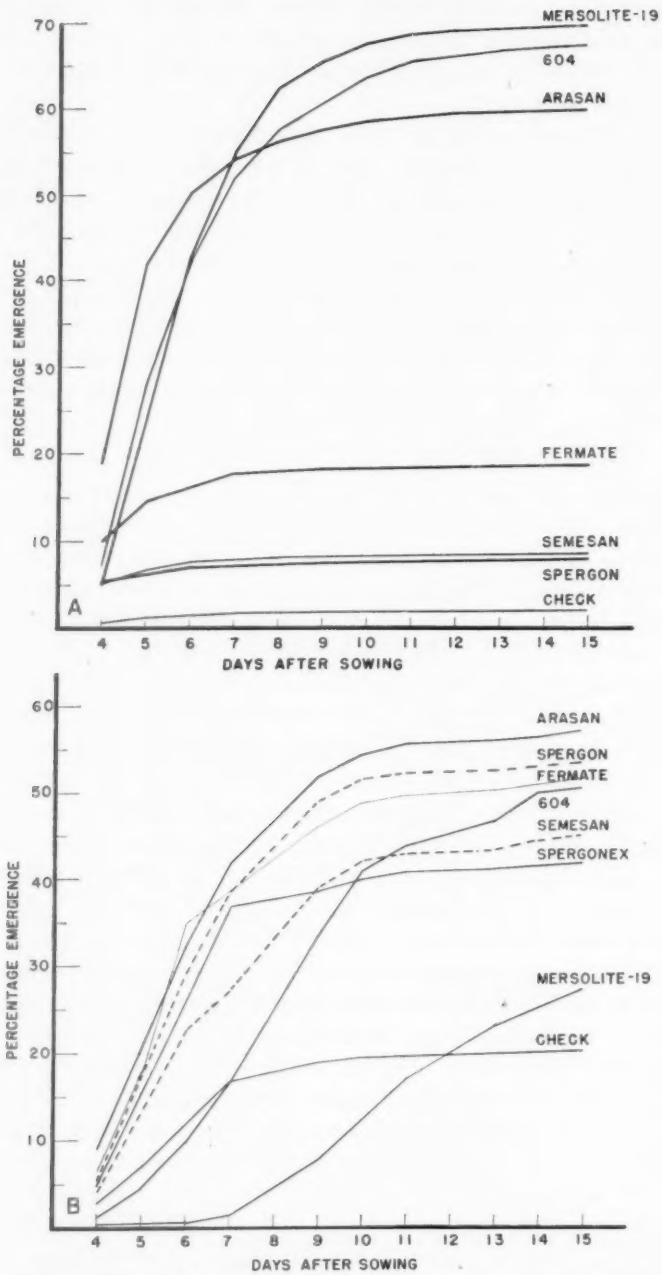


FIG. 1. Comparative effectiveness of fungicidal seed treatments and rate of guayule seedling emergence in nursery soil infested with damping-off fungi, mostly *Pythium ultimum*. A. Threshed seed. The emergence curve for Spergonex was practically the same as that of the check and for this reason was omitted. B. Unthreshed seed pretreated with sodium hypochlorite. Note the difference in response to the different fungicides on the two kinds of seed.

hypochlorite pretreatment would give effective pre-emergence protection against damping-off fungi and to compare its protective value with that of Arasan and No. 604. The results indicated that the sodium hypochlorite treatment did give some apparent protection against pre-emergence losses but that the increase in emergence was considerably less than that secured by the use of either Arasan or No. 604. Seedling emergence in fungus-infested soil was significantly better for threshed seed which had been treated with Arasan and No. 604 than for threshed seed which had received only the hypochlorite treatment. However, with hypochlorite-treated seed damping-off control was better when the seed was dusted with Arasan than with No. 604. On the other hand, in pasteurized soil the dusting of threshed hypochlorite-treated seed with No. 604 caused a significant decrease in emergence. This reduction in emergence was attributed to chemical injury. Sodium hypochlorite-treated seed appeared to be more sensitive to chemical injury than the raw seed. In this series of tests, as well as others, seedling emergence of threshed nonhypochlorite-treated seed in pasteurized soil was increased by dusting the seed with either Arasan or No. 604 (Table 1). The increases were so pronounced in some instances as to suggest the possibility of a stimulative action.

### NURSERY TESTS

Three seed protectants, Arasan, Spergon, and Semesan, which were procurable on the local market, were used in two trial nursery sowings in the Alisal Nursery in the spring of 1944. The treated seed were sown in plots of sufficient size to permit the use of regular nursery equipment. The seed was sown and covered with about one-fourth inch of sand by a seeder developed especially for the sowing of guayule seed. All cultural operations were comparable to standard nursery practices (1). The amount of water applied by the overhead sprinkler system and frequency of application were similar to those in the nurseries in 1943.

Seed used in the nursery tests were: (1) dry threshed seed which had not been pretreated with sodium hypochlorite; (2) dry unthreshed seed which had been pretreated with sodium hypochlorite and (3) soaked or pregerminated seed which had received the regular sodium hypochlorite pretreatment. The fungicides, dosage 1 per cent by weight, were applied as dust to the dry seed by means of a small rotary drum-type seed treater. In case of the soaked seed, the seed was first dusted with the fungicide and then moistened with tap water. Twice as much water by weight was used as seed. The dampened seed was thoroughly mixed and placed in open-top 5-gallon cans in the laboratory where it was kept for 4 or 5 days before sowing. During this soaking period the seed was stirred twice for aeration.

Only one kind of seed, threshed, dry unthreshed, or soaked unthreshed, was sown in a nursery section composed of 9 or 10 nursery beds 400 feet long. Each bed was divided into 4 equal plots, 100 feet long, and was treated as a randomized block which included each seed treatment. The first sowing was on April 30 and the second on May 20.

The daily average temperature during the critical period of 10 days following sowing ranged from about 55° to 60° F. For the same period the maximum temperature exceeded 78° F. on two days only and the minimum temperature did not fall much below 50° F. These temperatures were favorable, but somewhat lower than the optimum, for germination and emergence as indicated by greenhouse tests in which 70° to 80° proved to be more favorable for emergence than 65° or lower. Also, Benedict and Robinson (3) in laboratory tests found that optimum germination of guayule seed occurred at 68° to 77° F.

Emergence counts were made 2 or 3 times a week for 4 weeks following sowing in "count" plots which had been established at random before any seedlings had emerged. These plots consisted of a linear foot section of bed, 4 sq. ft. with 4 to a replication. In order to secure as accurate measure as possible of emergence, the seedlings were pulled out at time of counting.

### *Results of Treatment*

All three fungicidal seed treatments of dry threshed seed increased emergence over checks in both the April and May sowings (Table 2). In only one case was the increase not statistically significant at the end of 30 days, and this was near the boderline of significance. The emergence of threshed seed dusted with Arasan was better than for the other treatments. In the April (Table 2) sowing 79 per cent of the germinable seed dusted with Arasan had emerged by the 30th day after sowing, and 87 per cent of the viable seed in the May sowing.

In the May 20 sowing of dry unthreshed seed pretreated with sodium hypochlorite all treatments gave an increase in emergence over the check, but only Spergon gave a significant increase (Table 2). There was no significant difference in emergence between treatments at the end of 30 days in the April 30 sowing. No chemical injury was apparent with any of the treatments.

In the April 30 sowing, fungicidal seed treatments of soaked unthreshed seed pretreated with sodium hypochlorite did not significantly increase emergence (Table 2) over the control. In the May 20 sowing a significant increase in emergence was secured with both Arasan and Spergon. Seedling emergence from seed dusted with Semesan was sparse and scattered in both the April and May sowings and averaged less than 5 seedlings per square foot. Because of this failure, emergence counts of Semesan-treated seed are omitted from table 2. The deleterious action of Semesan apparently occurred during the 4-day period the seed was held in a moist condition prior to sowing.

The rate of emergence was somewhat more rapid in the April sowing than in the May sowing. In both cases emergence levelled off 18 to 20 days after sowing following a rather pronounced break about the 12th day. The difference in emergence between the two sowings, when based on viable seed sown, was not great enough to be considered important.

The inevitable loss which follows emergence occurred with all of the treatments in both the April and May sowings. There were no indications that the fungicidal seed treatments were effective in reducing post emergence losses. These losses (based on total emergence) ranged from 10 to 20 per cent for the different treatments at the end of 4 weeks after sowing. In a seedling density count made 8 weeks after sowing, the average loss of emerged seedlings had increased from about 15 per cent at the end of 4 weeks

TABLE 2.—*Effect of Arasan, Spergon, and Semesan on guayule seedling emergence in the nursery*

| Seed treatment | Germinable seed sown per sq. ft. | | Emerged seedlings per sq. ft.—30 days after sowing | | | |
|---|---|---|---|---|---|---|
| | | | Sown April 30[a] | | Sown May 20[b] | |
| | April 30 | May 20 | Total | Percentage of germinable seed | Total | Percentage of germinable seed |
| | *Number* | *Number* | *Number* | *Per cent* | *Number* | *Per cent* |
| Dry threshed seed | | | | | | |
| Arasan | 76 | 68 | 60.37 | 79.43 | 59.29 | 87.19 |
| Spergon | 76 | 68 | 46.33 | 60.96 | 43.96 | 64.65 |
| Semesan | 76 | 68 | 44.59 | 58.67 | 40.16 | 59.06 |
| Check | 76 | 68 | 40.51 | 53.30 | 19.33 | 28.43 |
| Dif. req. for sig. | ..... | ..... | 5.13 | ..... | 6.74 | ..... |
| Dry unthreshed hypochlorite-treated seed | | | | | | |
| Arasan | 81 | 91 | 40.05 | 49.44 | 59.31 | 65.18 |
| Spergon | 81 | 91 | 42.54 | 52.52 | 62.74 | 68.95 |
| Semesan | 81 | 91 | 44.05 | 54.38 | 60.45 | 66.43 |
| Check | 81 | 91 | 43.07 | 53.17 | 54.09 | 59.44 |
| Dif. req. for sig. | ..... | ..... | 3.23 | ..... | 7.99 | ..... |
| Soaked unthreshed hypochlorite-treated seed | | | | | | |
| Arasan | 89 | 73 | 39.53 | 44.42 | 40.21 | 55.08 |
| Spergon | 89 | 73 | 49.64 | 55.78 | 38.15 | 52.26 |
| Semesan | 89 | 73 | ..... | ..... | ..... | ..... |
| Check | 89 | 73 | 49.62 | 55.75 | 31.85 | 43.63 |
| Dif. req. for sig. | ..... | ..... | 5.86 | ..... | 4.75 | ..... |

[a] Ten replications of each treatment.
[b] Nine replications of each treatment.

to approximately 25 per cent 4 weeks later. These losses were caused by post-emergence damping-off, seedling root rot, soil splash, weeding, and insects. No attempt was made to evaluate the losses attributable to any one cause because of the difficulty involved in making accurate determinations.

## FIELD TESTS

The feasibility of guayule production by direct field sowing, as well as the production of nursery stock by furrow irrigation, has emphasized the importance of obtaining satisfactory seedling emergence and uniformity of stand under conditions different from that found in the nursery. Since

protectants were of benefit in increasing seedling emergence in the nursery and in the greenhouse, it was expected that similar results would be obtainable in subirrigated field sowings. However, it was realized that, while the conditions were similar in many respects in nursery and field sowings, there were also differences. In common nursery practice the seed is sown in beds, covered with sand and watered by an overhead sprinkler system, while in the field the seed is drilled in rows, covered with soil, and furrow irrigated. Because of these differences in sowing and watering it seemed probable that the germinating seed and emerging seedling would be subjected to greater pre-emergence hazards in field sowings than in the nursery.

Subirrigation plot tests were made during the summer of 1944 to ascertain the effectiveness of seed protectants in direct field sowings. Dry seed, either threshed or unthreshed, from two seed lots of strain 593, either 35–G or B–38, collected in 1943 were used. Only seed which had not been pretreated with sodium hypochlorite was treated with fungicides but the standard sodium hypochlorite treatment was included as one of the treatments. The check or control received no chemical treatment.

The seed was sown with a Planet Jr. seeder in an open V-type furrow 1 to 1½ inches deep and 2 to 4 inches from the edge of a shallow irrigation furrow. An effort was made to cover the seed with a minimum amount of soil. The rear press wheel was removed from the seeder and the seed was covered only with the soil that incidentally rolled or drifted onto the seed from the sides of the furrow. In this manner the seed was sown at the bottom of the furrow on firm or fairly compact soil and covered with ⅛ to ¼ inch of loose soil in 30-foot row plots.

The plots were furrow irrigated every other day for 2 to 3 hours. However, this schedule was sometimes varied, depending upon weather conditions and obvious need of irrigation. An effort was made to keep the seed sufficiently moist for optimum germination and emergence rather than to follow a predetermined irrigation schedule.

Frequent emergence counts were made starting with the earliest emergence and continuing up to 14 or 15 days after sowing. The center 15 feet of the 30-foot plots were used for emergence counts.

The air and soil surface temperatures were generally favorable for germination and emergence during July and August. The average air temperature during the two months was around 60° F., except for a few days in the third week of August when it was slightly lower. The soil temperature at a depth 0.5 to 1.0 inch averaged 10° F. higher than the air temperature.

### *Results*

In this series of experiments both the Arasan and No. 604 treatments increased emergence significantly over the hypochlorite treatment in both threshed and unthreshed seed (Table 3). Emergence in the check was uniformly poor in all cases. Seed dusted with No. 604 gave a significantly higher emergence than threshed seed treated with Arasan. On unthreshed

TABLE 3.—*Effect of fungicidal seed treatments on guayule seedling emergence in furrow irrigation tests*[a]

| Treatment | Emerged seedlings | | | | |
|---|---|---|---|---|---|
| | Threshed seed | | | Unthreshed seed | |
| | July 13[b] | July 13 | Aug. 2 | July 13 | Aug. 2 |
| | *Per cent*[c] | *Per cent*[c] | *Per cent*[c] | *Per cent*[c] | *Per cent*[c] |
| Arasan | 19.36 | 25.29 | 26.21 | 36.15 | 57.29 |
| No. 604 | 34.10 | 35.67 | 50.60 | 42.43 | 66.51 |
| Hypochlorite | 6.94 | 9.96 | 12.18 | 13.03 | 34.78 |
| Check | 2.40 | 0.92 | 3.60 | 0.47 | 1.29 |
| Difference required for significance (5 per cent) | 8.22 | 8.20 | 7.91 | 13.49 | 8.63 |

[a] Emergence is based upon all seedlings which had emerged within 15 days after sowing in the middle 15 feet of a 30-foot row plot.

[b] Date sown. Two different lots of threshed seed were sown on July 13; 35–G and B–38.

[c] Percentage of germinable seed sown.

seed the emergence was higher for the No. 604 treatment, although it was significantly so in only one of two instances.

## SUMMARY AND CONCLUSIONS

Guayule (*Parthenium argentatum* Gray) was found to be very susceptible to attack by pre-emergence damping-off fungi in nurseries and in direct field sowings at Salinas, California. Because of pre-emergence losses extensive resowings of nursery beds were necessary in 1943. Post-emergence damping-off losses were small compared with pre-emergence losses.

In greenhouse tests with 8 fungicides to determine their relative effectiveness in protecting guayule seedlings against pre-emergence losses from *Pythium* and *Rhizoctonia,* damping-off fungi, the optimum dosage was about 1 per cent by weight for Arasan, Spergon, Spergonex, Cuprocide, and Semesan; 0.5 to 0.75 per cent for No. 604; and 0.25 per cent or less for Mersolite-19.

Treating guayule seed with sodium hypochlorite increased seedling emergence in soil infested with *Pythium* and *Rhizoctonia* in the greenhouse as well as in sub-irrigated field-sown tests. However, when seed which germinated readily was dusted with Arasan or No. 604, emergence was higher than from hypochlorite-treated seed when sown in fungus-infested soil. The differences in protective effect were significantly in favor of the seed protectants over sodium hypochlorite.

When used on both threshed and unthreshed non-hypochlorite-treated seed in the greenhouse Arasan, No. 604, and Mersolite-19 gave the greatest increases in seedling emergence in fungus-infested soil. On unthreshed seed, which had been pretreated with sodium hypochlorite, Mersolite-19 did not increase emergence as much as Arasan and No. 604.

Under conditions favorable for germination in the greenhouse, guayule seedlings started to emerge on the 4th day and emergence was practically completed in 10 to 12 days after sowing.

On dry, threshed, non-hypochlorite-treated seed sown in the nursery, Arasan, Spergon, and Semesan increased emergence over the untreated seed in sowings made in April and May. With threshed guayule seed the treatment with Arasan resulted in more emerged seedlings than either Spergon or Semesan treatment.

Dry unthreshed seed that had been pretreated with sodium hypochlorite, when dusted with Arasan, Spergon, or Semesan gave an increase in emergence over the check in a sowing made in May. In an April sowing the differences in emergence for the seed dusted with these seed-protectants were not significant. Seed similarly treated, but soaked for 4 days prior to sowing, gave no increased emergence for any of the materials in the April sowing. In the May sowing a significant increase in emergence was secured with both Arasan and Spergon. In both sowings the emergence from seed dusted with Semesan was sparse, scattered, and considerably lower than for the check.

In a subirrigated field both Arasan and No. 604 increased seedling emergence significantly above the hypochlorite treatment and above non-hypochlorite-treated checks for both threshed and unthreshed seed. Emergence of threshed seed dusted with No. 604 was better than when Arasan was used.

Based on tests in greenhouse, nursery, and subirrigated field Arasan and No. 604 are considered as the two most promising of the several seed protectants tested in the control of pre-emergence damping-off of guayule. Although tested only in the greenhouse, Mersolite-19 gave excellent results.

SPECIAL GUAYULE RESEARCH PROJECT,
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# BREEDING FOR RESISTANCE TO EARLY BLIGHT IN THE IRISH POTATO[1]

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Early blight, caused by *Alternaria solani* (Ellis and G. Martin), L. R. Jones and Grout, does not usually occur in epiphytotic form, although occasionally it may be almost as destructive as late blight. Coons[4] stated that the annual loss in Michigan was about 25 per cent. According to Jones,[5] the yield may be reduced 10 to 25 per cent in Wisconsin. Several cases are on record of unusually severe attacks; more important, however, is the smaller but yearly toll of the disease.

No concerted effort has been made to breed new varieties of potatoes that are resistant to the pathogen causing early blight, although it has been known for many years that varieties of cultivated potatoes have different degrees of susceptibility. The early work of Stuart[6] in Vermont indicated that a few foreign varieties possessed some resistance to the fungus.

In 1940 work was started toward finding resistant seedlings or commercial varieties that could be used in a breeding program. The data herein are presented as a preliminary report.

## METHODS AND MATERIALS

Conditions favorable for a natural epiphytotic were not present during the growing seasons of 1940 to 1943, inclusive. The laying of a pipe line in 1943 permitted the use of supplemental water as an overhead spray on experimental plots to make conditions more favorable for infection and subsequent spread of the fungus. By the spring of 1945 methods had been developed so that a heavy infection was produced and it was possible to ascertain the resistance or susceptibility of a number of seedlings and varieties.

In this experiment the seedlings and varieties were grown adjacent to a row of the Houma variety, which is very susceptible to the organism.

The infection data were recorded in 4 classes as illustrated in figure 1:

Class 0 = No infection.

Class 1 = Light infection; spots few to many, small, not coalescing; foliage remained green.

[1] Cooperative investigations by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture and the Louisiana Agricultural Experiment Station.

[2] Pathologist; U. S. Department of Agriculture, Baton Rouge, Louisiana.

[3] The writer takes pleasure in acknowledging his indebtedness to Dr. F. J. Stevenson for furnishing many of the seedlings and many of the parents used in crosses that were included in this investigation.

[4] Coons, G. H. Potato diseases of Michigan. Mich. Agr. Exp. Sta. Special Bull. 66. 1914.

[5] Jones, L. R. Potato diseases in Wisconsin and their control. Wis. Agr. Exp. Sta. Circ. 36. 1912.

[6] Stuart, William. Disease resistance of potatoes. Third report. Vt. Agr. Exp. Sta. Bull. 179: 147–183. 1914.

Class 2 = Moderate infection; spots few to many, medium to large, some coalescing; foliage 0–25 per cent killed.

Class 3 = Severe infection; spots many, medium to large, coalescing; foliage burned, 26–100 per cent killed.

Conditions very favorable for infection prevailed during all of May, 1945. By May 3 most of the leaves were killed on the Houma checks and many of the seedlings and some varieties were severely infected.

Because of differences in maturity of the material being tested, notes were taken on several dates.

In this test, 19 named varieties and 445 numbered seedlings were exposed to infection. Each of these was grown in units of at least 5 hills. The seedlings represented many different crosses and a few inbred parents. Since no segregating progenies were included, it is not possible to draw conclusions as to the mode of inheritance of resistance to the early-blight fungus.

## RESULTS

The reactions of 19 commercial varieties of potatoes to early blight are in table 1. All but 2 of these varieties were very susceptible to the fungus. Of the 2, Sequoia was moderately infected, but the infection in Menominee was only slight. Menominee also possesses a high degree of resistance to *Actinomyces scabies* and moderate resistance to *Phytophthora infestans*. The early-maturing varieties—Cobbler, Earlaine, Red Warba, Triumph, and Warba—were severely infected early in May whereas some of the later-maturing sorts were not infected to the same degree until 2 weeks later. The data also demonstrate that lateness is not completely correlated with

TABLE 1.—*Progressive development on 19 commercial varieties of Irish potatoes of infection by the early-blight fungus in the field at Baton Rouge, Louisiana, in 1945*

| Variety | Incidence of disease[a] on | | | |
|---|---|---|---|---|
| | May 3 | May 5 | May 18 | June 2 |
| Chippewa | 0 | 3 | 3 | 3 |
| Cobbler | 3 | 3 | 3 | 3 |
| Desoto | .... | .... | .... | 3 |
| Earlaine | 3 | 3 | 3 | 3 |
| Earlaine No. 2 | 0 | 0 | 2 | 3 |
| Golden | 0 | 0 | 3 | 3 |
| Green Mountain | 0 | 3 | 3 | 3 |
| Kasota | 0 | 3 | 3 | 3 |
| Katahdin | 0 | 0 | 3 | 3 |
| LaSalle | .... | .... | .... | 3 |
| Menominee | 0 | 0 | 1 | 1 |
| Mesaba | 3 | 3 | 3 | 3 |
| Mohawk | 0 | 3 | 3 | 3 |
| Pontiac | 3 | 3 | 3 | 3 |
| Red Warba | 3 | 3 | 3 | 3 |
| Sebago | 0 | 0 | 3 | 3 |
| Sequoia | 0 | 0 | 2 | 2+ |
| Triumph | 3 | 3 | 3 | 3 |
| Warba | 3 | 3 | 3 | 3 |

[a] Infection classes; 0 = no infection, 1 = light, 2 = moderate, 3 = severe.

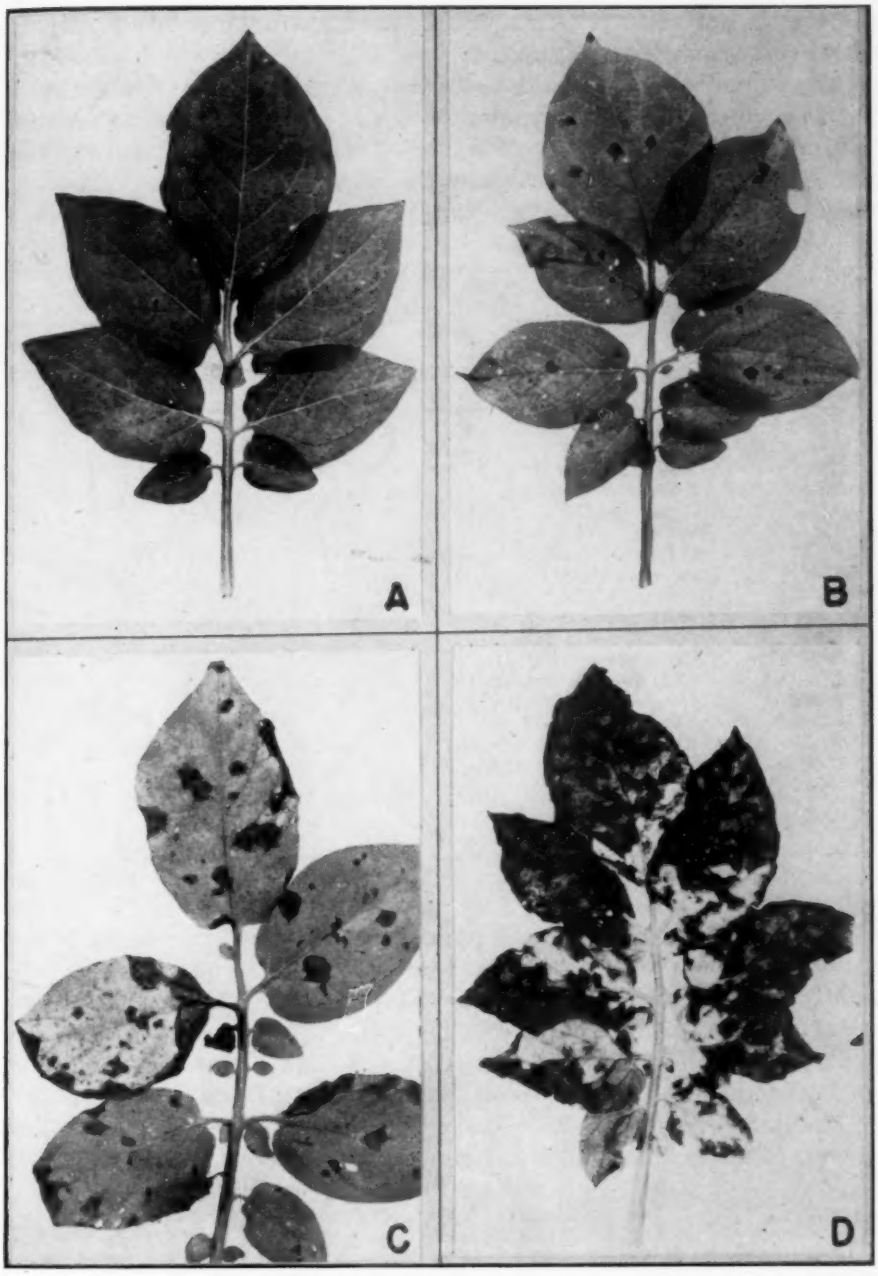


Fig. 1. Infection classes: A = no infection (class 0); B = light infection (class 1); C = moderate infection (class 2); D = severe infection (class 3).

early-blight resistance since the late-maturing and very-late-maturing varieties, such as Earlaine No. 2, Katahdin, Mohawk, and Pontiac, were killed by the fungus before they matured.

The 20 seedlings listed in table 2 were selected to represent the various groups of infection types that were found among the 445 seedling varieties tested. Seedlings XL 127–2 and XL 154–7 were severely infected early in May and both of these are early-maturing sorts. Infection was delayed in many other seedlings listed in table 2.

TABLE 2.—*Progressive development on some seedling varieties of Irish potatoes of infection by the early-blight fungus in the field at Baton Rouge, Louisiana, in 1945*

| Pedigree | Parentage | Incidence of disease[a] on | | | |
|---|---|---|---|---|---|
| | | May 3 | May 5 | May 18 | June 2 |
| XL 127–2 | 96–140 × 926–36 | 3 | 3 | 3 | 3 |
| XL 154–7 | 47562 × 47156 | 3 | 3 | 3 | 3 |
| XL 28–1 | 336–123 × 336–18 | 0 | 0 | 2 | 3 |
| XL 29–23 | Katahdin × Earlaine | 0 | 0 | 2 | 2 |
| XL 133–9 | Sebago × 47156 | 0 | 0 | 1 | 3 |
| XL 133–6 | Sebago × 47156 | 0 | 0 | 1 | 2– |
| X 512–1 | WI–6 × Katahdin | 0 | 0 | 1 | 2– |
| X 987–1 | 792–78 × Katahdin | 0 | 0 | 1 | 2– |
| B 94–3 | 336–144 × 47562 | 0 | 0 | 1 | 2– |
| B 70–5 | B 127 × 96–56 | ..... | ..... | ..... | 3 |
| XL 148–1 | 528–229 × 528–170 | ..... | ..... | ..... | 1+ |
| XL 8–61 | Chippewa × 47156 | ..... | ..... | ..... | 1 |
| XL 211–1 | (XL 89–1) × (XL 72–1) | ..... | ..... | ..... | 1 |
| XL 306–2 | 499–a × B 56–11 | ..... | ..... | ..... | 1 |
| XL 308–5 | Pontiac × B 44–14 | ..... | ..... | ..... | 1 |
| X 528–118 | Richter's Jubel × 44537 | ..... | ..... | ..... | 1 |
| X 627–103 | Hindenberg × Katahdin | ..... | ..... | ..... | 1 |
| B 69–12 | Katahdin × 96–56 | ..... | ..... | ..... | 1 |
| B 273–31 | President × 96–28 | ..... | ..... | ..... | 1 |
| B 273–39 | President × 96–28 | ..... | ..... | ..... | 1 |

[a] Infection classes; 0 = no infection, 1 = light, 2 = moderate, 3 = severe.

From the standpoint of breeding for resistance to the early-blight pathogen it is significant that a number of seedlings were found that were only slightly infected. Several of these are listed in table 2. Four of these seedlings (XL 211–1, XL 306–2, X 528–118, and X 627–103) are moderately to highly resistant to the scab organism. A very high degree of resistance to the late-blight fungus occurs in B 273–31 and B 273–39. Seedlings XL 148–1, XL 8–61, and B 69–12 are very vigorous and produce high yields of tubers. Seedling XL 308–5 is red-skinned and vigorous.

## SUMMARY AND CONCLUSIONS

A large-scale, field test was made in 1945 at Baton Rouge, Louisiana, to test the reactions of 19 named varieties and 445 seedling varieties to the early-blight fungus (*Alternaria solani*).

The results failed to demonstrate the presence of immunity. A number of the seedlings were only slightly affected whereas the greater part of them

were moderately to severely affected. Of 19 named varieties tested, only Menominee had any marked degree of resistance.

Among the seedling varieties only slightly affected by the *Alternaria*, 4 are also moderately to highly resistant to the scab organism, 2 possess a high degree of resistance to the late-blight fungus, 3 are very vigorous and produce high yields of tubers, and one is red-skinned and vigorous.

Thus, from the standpoint of breeding for resistance to early blight a number of promising seedling varieties are at hand that can be used in future pollination work. In addition, some of these seedlings also possess genetic factors for resistance to the pathogens causing scab and late blight as well as factors for yielding ability. Hence it should not be very difficult to produce varieties combining resistance to early blight with resistance to other diseases as well as with other characters of commercial importance.

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# LEAFROLL NET NECROSIS AND STEM-END BROWNING OF POTATO TUBERS IN RELATION TO TEMPERATURE AND CERTAIN OTHER FACTORS

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## INTRODUCTION

A crop from a potato (*Solanum tuberosum* L.) field has no potentiality for the development, in storage, of leafroll net necrosis unless at least three conditions prevail. First, the variety must be susceptible to the leafroll virus. Second, leafroll must have infected plants, during the growing season, that were healthy at the beginning of the season. Third, the variety must be one that can develop net necrosis as a symptom of recent infection by leafroll. However, even when having the potentiality resulting from the fulfillment of these three conditions, crops, parts of crops, and tubers have been observed which either did not develop net necrosis in storage or developed much less than was to be expected. Another type of internal discoloration, called stem-end browning, was found commonly in the Maine potato crops of 1923, 1927, 1929, and 1937 and was differentiated from net necrosis (3, 4). This paper is concerned with the effects of temperature, length of storage period, size of tuber, and certain other factors, upon the development of net necrosis and stem-end browning in storage.

In 1940 the cause of stem-end browning was still undetermined and attempts were made, as they had been previously, to isolate organisms from affected tubers. Isolations were unsuccessful in the majority of cases, and none of the miscellaneous lot of organisms obtained produced any stem-end browning or other change when inoculated into healthy tubers (10, p. 503).[1] In the winter of 1942–43, steam sterilization of soil from fields that had produced stem-end browning crops did not reduce stem-end browning in the greenhouse crop of plants grown in the sterilized soil, in comparison with greenhouse crops in unsterilized soil from the same fields (13, p. 232). About 180 hills were dug individually in 1943 in 6 fields. Field notes were taken on stem infection by *Rhizoctonia solani* and, later, storage records were taken on the development of stem-end browning at about optimum temperature. In general "very slight" Rhizoctonia infection was followed by the highest percentages of stem-end browning, but the most stem-end browning was in the tubers from "healthy" hills in the case of one field, and in the tubers from "severely infected" hills in the case of another field.

Recently it has been determined by Ross (14, p. 530–531) that stem-end browning in Maine can become, under suitable storage conditions, much more prevalent in one strain of the Green Mountain variety than it can in other strains of the same variety in the same environment. Ross (14, p.

[1] Data are given in considerable detail in the Maine Agricultural Experiment Station report bulletins referred to in this paper.

529–530) has further transmitted this potentiality from one tuber line or plant to another by grafting. His data indicate that a virus is one factor in the development of stem-end browning.

Small-scale preliminary tests with Green Mountain tubers of the crop of 1937 disclosed a tendency for more internal discoloration to develop at 35°, 46°, and 53° F. than at 71°, 86°, or 96°, at constant temperature, or than at a variable temperature of 32° to 64°, averaging 43° (9, p. 800). Similarly, a series of Green Mountain samples of the 1938 crop developed net necrosis in 0, 11, 13, 0, 0.1, and 0 per cent of the tubers when kept at 40°, 42°, 56°, 76°, 83°, and 70° to 90° F. respectively (9, p. 800). Another 1938 series at these same temperatures developed respectively 0, 1.9, 1.3, 0.3, 0.4, and 0 per cent net necrosis and 0, 1.9, 3.2, 0.7, 0.7, and 0 per cent stem-end browning (9, p. 800).

These preliminary tests indicated an optimum constant-temperature range of about 42° to 56° F. for both net necrosis and stem-end browning. They also indicated that such temperature tests require many identical samples of two to three bushels each.

More samples of larger size, composed to be identical, were taken from the crop of 1939 and stored in seven places, with results as follows:

| Temperature, in °F. | 32 | 37 | 41 | 45 | 54 | 72 | 77 | 86 |
|---|---|---|---|---|---|---|---|---|
| Net necrosis, in per cent | 0 | 2 | 3 | 1 | 4 | 1 | 0 | 0 |
| Stem-end browning, in per cent | 1 | 11 | 9 | 6 | 9 | 2 | 1 | 0 |

Here the 45° F. storage had a fluctuating temperature that, with an average near optimum as judged by results in constant-temperature storages, was nevertheless less conducive than they were to the development of internal discoloration, just as was the case in the 1937 crop. Apparently it is the number of hours near optimum temperature that counts, more than the average temperature.

### METHODS WITH CROPS OF 1940 TO 1945

Twelve experimental storage bins each with a capacity of about 100 barrels (a "barrel" being 165 lb.) were constructed at Aroostook Farm, near Presque Isle, Maine, in the summer of 1940 (10, p. 498). These bins were equipped with cooling apparatus, heaters, portable calcium chloride driers, and sprinkling apparatus for the maintenance of temperatures from 30° F. up and for the partial control of humidity. Samples of a crop were shifted from one bin to another if a change of temperature or humidity was desired. All samples of any crop were made up and examined by the same operator.[2]

Unless stated to be otherwise, samples were of the Green Mountain variety and were made up in sets of 36 from each field as follows. Thirty-six field-run barrels of tubers were spread out, a barrel at a time. From each barrel the 36 apparently largest tubers were distributed to the 36 samples. This was repeated until the contents of the barrel were completely

[2] Frank Upton, in crops of 1940 and 1941, and Michael Goven, in crops of 1942, 1943, and 1945.

divided. Each barrel was taken in turn so that the end result was 36 composite samples each containing 1/36 of each original field-run barrel and all practically identical as to tuber sizes. When duplicates were kept in the same bin for the same length of time, the percentage of tubers with net necrosis or stem-end browning was practically the same, so that each series of 36 samples could be used for comparing 36 different combinations of temperature, duration, and humidity. No method was found successful for making more than one examination of a given sample.

### EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY

#### *Results with the Crop of 1940*

Tests were made with 432 samples (12 series of 36 each) from the crop of 1940 from four fields. There was the least of either kind of internal discoloration at 32–33° F., the most at 50°, and intermediate percentages at 38° and 60°. Several comparisons by Student's method were possible, each between 22 to 24 samples in one bin and the same number in another bin, the members of each pair coming from the same series of 36 composite samples made up at harvest time. The results, given in table 1, show in comparisons 1, 4, and 5 that 5 to 12 degrees' difference gave highly significant differences in the percentage of tubers with net necrosis or stem-end browning; in the other comparisons, with little or no difference in temperature there were only nonsignificant differences in internal discoloration. The

TABLE 1.—*Effect of temperature differences on percentage of tubers having net necrosis and developing stem-end browning in storage. Samples compared by Student's method.*[a] *Crop of 1940*

| Comparison | Pairs[b] | Temperature | Relative humidity | Net necrosis | | Stem-end browning | |
|---|---|---|---|---|---|---|---|
| | | | | Means | Odds[c] | Means | Odds[c] |
| | | °F. | *Per cent* | *Per cent* | | *Per cent* | |
| 1 | 22 | 33 | 86 | 3.33 | 128: 1 | 1.14 | 61: 1 |
| | | 38 | 89 | 6.61 | | 1.75 | |
| 2 | 22 | 38 | 77 | 4.88 | 14: 1 | 1.44 | Under 10: 1 |
| | | 38 | 89 | 6.61 | | 1.75 | |
| 3 | 22 | 39 | 79 | 8.42 | 3.67: 1 | 2.40 | Under 2: 1 |
| | | 38 | 89 | 7.81 | | 2.32 | |
| 4 | 24 | 38 | 89 | 5.36 | Over 9999: 1 | 2.12 | Over 9999: 1 |
| | | 50 | 88 | 10.60 | | 13.29 | |
| 5 | 22 | 39 | 79 | 8.42 | Over 3332: 1 | 2.40 | Over 9999: 1 |
| | | 50 | 82 | 17.67 | | 14.12 | |
| 6 | 22 | 50 | 75 | 17.27 | Under 3: 1 | 13.44 | Under 10: 1 |
| | | 50 | 82 | 17.67 | | 14.12 | |
| 7 | 23 | 60 | 71 | 6.78 | Under 10.5: 1 | 10.99 | Under 6.52: 1 |
| | | 60 | 79 | 6.10 | | 10.39 | |

[a] Method as given in (8).

[b] In each comparison, 22 to 24 samples, of 700 to 1000 tubers apiece, held in one storage locker were paired respectively with the same number of samples in another storage locker, the members of each pair having come from the same series of 36 composite samples and having been stored for the same length of time.

[c] Odds considered significant only if 30 or more to 1.

relative humidity (71 to 89 per cent) had no apparent effect on either kind of defect. At 50° F., stem-end browning continued to increase appreciably until the middle of November, and net necrosis usually until in December or January but until in March or April in some series of samples. Samples kept at low temperatures until in November and then warmed to the optimum temperature developed net necrosis but not stem-end browning, indicating a permanent inhibition of stem-end browning by exposure to low temperature during the first part of the storage season.

*Results with the Crop of 1941*

No attempt was made to control humidity after the tests with the crop of 1940, although relative humidity was recorded daily. The 288 samples (8 series of 36 each) of the crop of 1941 from 4 fields were variously exposed to temperatures of 33°, 37°, 40°, 44°, 47°, 51°, and 60° F. The optimum tested temperatures for the development of net necrosis were 44°, 47°, and 51° F., respectively, for the three series having an appreciable amount of the defect, running about 33, 28, and 37 per cent (11, p. 298–302, 329). In the case of stem-end browning the percentage of affected tubers was greater at 60° F. (running from 9 to 47 per cent) than at lower temperatures. The maximum percentage was reached or nearly reached in 45 to 65 days after harvesting.

Some samples were shifted from suboptimum to optimum temperatures. Samples held 10 to 30 days at 33° F. and then moved to 47° developed less net necrosis than samples held from the first at 47° in the same period of time. Further, 30 to 90 days at 33° apparently conditioned the tubers so that subsequent exposure to 47° was followed by no further development, or very little, of net necrosis. In one series of samples, stem-end browning developed in 120 days in 13 per cent of the tubers stored at 33°, in 33 per cent at 51°, in 14 per cent at 33° for 60 days followed by 51° for 60 days, and in 23 per cent at 33° for 30 days followed by 51° for 90 days. Apparently 60 days at 33° F. "conditioned" the tubers so that subsequent exposure to 51° was followed by practically no further development of stem-end browning.

*Results with the Crop of 1942*

One hundred forty-four samples (4 series of 36 each) from four fields, crop of 1942, were variously exposed to storage temperatures of 31°, 33°, 36°, and 51° F. at which the relative humidity averaged, respectively, about 76, 75, 78, and 78 per cent. The maximum percentage of tubers with net necrosis was reached, or practically reached, at 52° F., in 60 to 90 days after digging, and, with stem-end browning, in 60 to 120 days after digging. Samples kept until 200 days after digging showed, in some series, some decrease from the maximum in percentage net necrosis or with stem-end browning at both 51° and 36° F. (13, p. 279). The data in table 2 show that considerably more net necrosis and stem-end browning developed in 90 days in storage at 51° F. than at 31°, 33°, or 36°. The difference was

somewhat less in the samples from Field 3 where about 12 per cent of the tubers already had net necrosis at harvest time.

Some samples were exposed first to suboptimal or supraoptimal temperatures and then to about optimum temperature. The data, in table 3, show that 60 days' "conditioning" at 31°, 33°, 36°, or 71° F. reduced considerably the amount of net necrosis or stem-end browning that developed subsequently in 60 days at 51° F., in stocks where the discoloration potential was present, as compared with the development in 60 to 120 days at a constant temperature of 51°. The permanently inhibiting effect of extreme temperatures was somewhat less in the samples from Field 3 where net necrosis had already developed in 12 per cent of the tubers by harvest time. In the other series of samples the suppressing effect of low temperature was

TABLE 2.—*Development of net necrosis and stem-end browning in samples of 1942 crop kept for 90 days at several constant temperatures*

| Field | Net-necrosis percentage | | | | Stem-end browning percentage | | | |
|---|---|---|---|---|---|---|---|---|
| | 31° F. | 33° F. | 36° F. | 51° F. | 31° F. | 33° F. | 36° F. | 51° F. |
| 1 | 0 | 0 | 0 | 0.4 | 0.6 | 0.7 | 1.7 | 21.7 |
| 2 | 0 | 0 | 0 | 0.1 | 1.1 | 1.3 | 1.6 | 39.5 |
| 3 | 15.3 | 17.1 | 20.0 | 36.2 | 1.4 | 1.5 | 1.5 | 6.4 |
| 3[a] | 3.4 | 5.2 | 8.1 | 24.3 | ..... | ..... | ..... | ..... |
| 4 | 0.4 | 0.6 | 0.7 | 15.0 | 0.7 | 0.7 | 0.4 | 15.9 |

[a] Increase over the 11.9 per cent at harvest time, when storage at different temperatures was begun.

somewhat greater on stem-end browning than on net necrosis, and high temperature inhibited net necrosis somewhat more than low temperature did.

### *Results with the Crop of 1943*

One hundred forty-four samples (4 series of 36 each) from four fields, crop of 1943, were variously exposed to storage temperatures of 32°, 36°, 70°, 65°, and 50° F. at which the relative humidity averaged, respectively, about 79, 78, 78, 78, and 87 per cent. At 50°, the maximum percentage of tubers with net necrosis was reached in 90 to 100 days and the percentage then gradually declined until in April when the tubers were too rotted, sprouted, and shrivelled for accurate diagnosis. At 50°, the maximum percentage of tubers with stem-end browning was not reached in two series until 120 to 240 days after harvest time, with no subsequent decline. Some samples, listed in table 4, showed that conditioning at 70° or 32° F. for 60 days, previous to 60 days at 50° F., completely prevented or greatly reduced the development of net necrosis and stem-end browning. Conditioning at 65° or 36° was less effective in some instances. Other samples in the same series (13, p. 278) showed that at 70° and 65° shorter periods of conditioning (30 and 15 days) were often much less effective than was conditioning for 60 days.

Field 2 had been in potatoes the past three years, was on the same farm as Field 2 of 1941 and Field 1 of 1942 in these studies, and was expected to

yield a crop with as much stem-end browning as these other fields had. The drop to practically none can be attributed to a change in the source of seed,

TABLE 3.—*Net necrosis and stem-end browning in samples of 1943 crop and the permanently inhibiting effect of low or high temperature during first part of storage*

| Field | Days in storage[a] at | | | | | Net necrosis | Stem-end browning |
|---|---|---|---|---|---|---|---|
| | 31° F. | 33° F. | 36° F. | 71° F. | 51° F. | | |
| | | | | | | *Per cent* | *Per cent* |
| 1 | | | | | 60 | 0.3 | 16.2 |
| | 60 | | | | 60 | 0.0 | 0.4 |
| | | 60 | | | 60 | 0.0 | 0.4 |
| | | | 60 | | 60 | 0.0 | 0.9 |
| | | | | | 120 | 0.3 | 22.1 |
| 2 | | | | | 60 | 0.2 | 30.6 |
| | 60 | | | | 60 | 0.0 | 0.3 |
| | | 60 | | | 60 | 0.1 | 0.5 |
| | | | 60 | | 60 | 0.0 | 1.2 |
| | | | | | 120 | 1.7 | 42.1 |
| 3 | | | | | 60 | 29.7 | 5.2 |
| | 60 | | | | 60 | 18.7 | 2.2 |
| | | 60 | | | 60 | 17.1 | 1.6 |
| | | | 60 | | 60 | 26.6 | 3.3 |
| | | | | | 120 | 35.3 | 5.7 |
| 3[b] | | | | | 60 | 17.8 | ..... |
| | 60 | | | | 60 | 6.8 | ..... |
| | | 60 | | | 60 | 5.2 | ..... |
| | | | 60 | | 60 | 14.7 | ..... |
| | | | | | 120 | 23.4 | ..... |
| 4 | | | | | 60 | 15.8 | 11.1 |
| | 60 | | | | 60 | 1.0 | 0.0 |
| | | 60 | | | 60 | 2.2 | 0.4 |
| | | | 60 | | 60 | 4.7 | 1.5 |
| | | | | | 120 | 17.1 | 14.7 |
| 1 | | | | | 30 | 0.4 | 8.2 |
| | | | | 60 | 30 | 0.1 | 1.0 |
| | | | | | 90 | 0.4 | 21.7 |
| 2 | | | | | 30 | 0.0 | 23.3 |
| | | | | 60 | 30 | 0.0 | 0.9 |
| | | | | | 90 | 0.1 | 39.5 |
| 3 | | | | | 30 | 22.7 | 4.8 |
| | | | | 60 | 30 | 12.2 | 2.1 |
| | | | | | 90 | 36.2 | 6.4 |
| 3[b] | | | | | 30 | 10.8 | ..... |
| | | | | 60 | 30 | 0.3 | ..... |
| | | | | | 90 | 24.3 | ..... |
| 4 | | | | | 30 | 9.4 | 5.1 |
| | | | | 60 | 30 | 1.9 | 1.8 |
| | | | | | 90 | 15.0 | 15.9 |

[a] Periods in storage at different temperatures given from left to right as they occurred in the experiment.

[b] Increase over the 11.9 per cent at harvest time, when storage at different temperatures was begun.

the owner of the farm having been convinced that there was a difference between strains of Green Mountain with respect to the ability to develop stem-end browning.

TABLE 4.—*Net necrosis and stem-end browning in samples of 1943 crop and the permanently inhibiting effect of low or high temperature during first part of storage*

| Field | Days in storage[a] at | | | | | Net necrosis | Stem-end browning |
|---|---|---|---|---|---|---|---|
| | 32° F. | 36° F. | 70° F. | 65° F. | 50° F. | | |
| | | | | | | *Per cent* | *Per cent* |
| 1 | 60 | | | | 61 | 0.0 | 0.0 |
| | | 60 | | | 61 | 0.3 | 0.0 |
| | | | 60 | | 61 | 0.0 | 0.2 |
| | | | 30 | 30 | 61 | 0.0 | 8.6 |
| | | | | 60 | 61 | 0.0 | 34.6 |
| | | | | | 60 | 2.2 | 25.5 |
| | | | | | 121 | 1.1 | 30.0 |
| 3 | 60 | | | | 61 | 0.1 | 0.0 |
| | | 60 | | | 61 | 3.5 | 0.0 |
| | | | 60 | | 61 | 0.6 | 0.1 |
| | | | 30 | 30 | 61 | 2.8 | 0.0 |
| | | | | 60 | 61 | 7.8 | 5.5 |
| | | | | | 60 | 18.6 | 6.1 |
| | | | | | 121 | 17.6 | 4.5 |

[a] Periods in storage at different temperatures given from left to right as they occurred in the experiment.

### *Results with the Crop of 1945*

No study was made of these defects with the 1944 crop. However, 72 samples (2 series of 36 each) from the crop of 1945, from two fields, were exposed to storage temperatures of 34°, 38°, 44°, and 50° F. at which the relative humidity averaged, respectively, about 83, 83, 85, and 90 per cent. At 50° F. the maximum percentage of tubers with net necrosis was reached in 70 to 90 days and with stem-end browning in 100 to 130 days. Each kind of internal discoloration was graded in each tuber as either "cull" or "mild," the distinction being made on the basis of whether more or less than 5 per cent of the weight of the tuber had to be sliced off in order to remove all discoloration. This is a distinction used in the U. S. grades for potatoes, grade No. 1 having tolerance for only a certain amount of tubers with 5 per cent waste as determined by slicing. The several temperatures were considered to be representative of potato storages (2, Tables 1 and 5) and certain samples were shifted from suboptimum to optimum temperatures to simulate the succession of storage, transit, and market conditions in commercial practice.

As indicated in table 5, exposure to 50° F. for one or two weeks after exposure to a low temperature often resulted in considerably more net necrosis than developed in the same period during exposure to only the low temperature. For example, in sample 215 after 72 days at 34° F. there was only 3 per cent cull net necrosis while in sample 214 after 56 days at 34° and 15 days at 50° (total 71 days) there was 7 per cent cull net necrosis. However, a shift from 34° or 38° to 50° did not have a similar increasing effect upon stem-end browning, which apparently was permanently inhibited by these two lower temperatures more than was net necrosis. The

TABLE 5.—*Effect of storage temperature on net necrosis and stem-end browning in samples of 1945 crop*

| Sample[a] | Days in storage[b] at | | | | | Av. temp. | Tubers with | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 34° F. | 38° F. | 44° F. | 50° F. | All temp. | | Net necrosis | | Stem-end browning | |
| | | | | | | | Culle | Total | Culle | Total |
| | | | | | | °F. | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 102 | 28 | | | 8 | 36 | 38 | 1 | 2 | 0 | T[d] |
| 105 | | 28 | | 7 | 35 | 40 | 3 | 5 | 0 | 0 |
| 108 | | | 28 | 7 | 35 | 45 | 8 | 10 | 0 | T |
| 111 | | | | 35 | 35 | 50 | 10 | 13 | 1 | 6 |
| 104 | 43 | | | | 43 | 34 | 1 | 2 | 0 | 0 |
| 103 | 28 | | | 14 | 42 | 39 | 1 | 5 | 0 | 0 |
| 107 | | 43 | | | 43 | 38 | 2 | 3 | T | T |
| 106 | | 28 | | 14 | 42 | 42 | 3 | 7 | 0 | 0 |
| 110 | | | 43 | | 43 | 44 | 8 | 10 | 0 | T |
| 109 | | | 28 | 14 | 42 | 46 | 7 | 10 | 0 | 1 |
| 112 | | | | 42 | 42 | 50 | 10 | 17 | 2 | 6 |
| 113 | 56 | | | 8 | 64 | 36 | 1 | 3 | 0 | 0 |
| 116 | | 56 | | 8 | 64 | 40 | 3 | 5 | 0 | 0 |
| 119 | | | 56 | 8 | 64 | 45 | 8 | 10 | 1 | 5 |
| 122 | | | | 64 | 64 | 50 | 11 | 12 | 10 | 30 |
| 115 | 70 | | | | 70 | 34 | 1 | 3 | 0 | 0 |
| 114 | 56 | | | 14 | 70 | 37 | 1 | 5 | 0 | 0 |
| 118 | | 71 | | | 71 | 38 | 4 | 8 | 0 | 0 |
| 117 | | 56 | | 14 | 70 | 40 | 3 | 7 | 0 | 0 |
| 121 | | | 71 | | 71 | 44 | 9 | 11 | 1 | 2 |
| 120 | | | 56 | 14 | 70 | 45 | 8 | 9 | 1 | 3 |
| 123 | | | | 70 | 70 | 50 | 10 | 12 | 9 | 23 |
| 124 | 84 | | | 7 | 91 | 35 | 1 | 2 | 0 | 0 |
| 127 | | 84 | | 7 | 91 | 39 | 3 | 6 | 0 | 0 |
| 130 | | | 84 | 7 | 91 | 45 | 9 | 10 | 1 | 6 |
| 133 | | | | 91 | 91 | 50 | 10 | 10 | 14 | 32 |
| 126 | 100 | | | | 100 | 34 | 1 | 3 | 0 | 0 |
| 125 | 84 | | | 14 | 98 | 36 | 0 | 3 | 0 | 0 |
| 129 | | 99 | | | 99 | 38 | 4 | 6 | 0 | 0 |
| 128 | | 84 | | 14 | 98 | 40 | 4 | 6 | 0 | 0 |
| 132 | | | 99 | | 99 | 44 | 9 | 10 | 2 | 9 |
| 131 | | | 84 | 14 | 98 | 45 | 9 | 9 | 3 | 10 |
| 134 | | | | 98 | 98 | 50 | 10 | 10 | 16 | 36 |
| 135 | | | | 99 | 99 | 50 | 10 | 10 | 15 | 34 |
| 136 | | | | 126 | 126 | 50 | 8 | 8 | 17 | 34 |
| 202 | 28 | | | 9 | 37 | 38 | 4 | 8 | 0 | 0 |
| 205 | | 28 | | 9 | 37 | 41 | 7 | 13 | 0 | 0 |
| 208 | | | 28 | 10 | 38 | 46 | 23 | 26 | 0 | 0 |
| 211 | | | | 37 | 37 | 50 | 23 | 26 | T | T |
| 204 | 43 | | | | 43 | 34 | 2 | 5 | 0 | 0 |
| 203 | 28 | | | 15 | 43 | 40 | 4 | 10 | 0 | 0 |
| 207 | | 44 | | | 44 | 38 | 5 | 9 | 0 | 0 |
| 206 | | 28 | | 15 | 43 | 42 | 11 | 16 | 0 | 0 |
| 210 | | | 44 | | 44 | 44 | 22 | 24 | 0 | 0 |
| 209 | | | 28 | 16 | 44 | 46 | 22 | 25 | 0 | 0 |
| 212 | | | | 43 | 43 | 50 | 28 | 32 | 1 | 1 |
| 213 | 56 | | | 8 | 64 | 36 | 7 | 16 | 0 | 0 |
| 216 | | 56 | | 8 | 64 | 40 | 12 | 16 | 0 | 0 |
| 219 | | | 56 | 8 | 64 | 45 | 23 | 26 | T | 1 |
| 222 | | | | 64 | 64 | 50 | 31 | 32 | 2 | 3 |

TABLE 5 (*Continued*)

| Sample[a] | Days in storage[b] at | | | | | Av. temp. | Tubers with | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 34° F. | 38° F. | 44° F. | 50° F. | All temp. | | Net necrosis | | Stem-end browning | |
| | | | | | | | Cull[c] | Total | Cull[c] | Total |
| | | | | | | °F. | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 215 | 72 | | | | 72 | 34 | 3 | 5 | 0 | 0 |
| 214 | 56 | | | 15 | 71 | 37 | 7 | 14 | 0 | 0 |
| 218 | | 72 | | | 72 | 38 | 7 | 12 | 0 | 0 |
| 217 | | 56 | | 15 | 71 | 41 | 14 | 18 | 0 | 0 |
| 221 | | | 72 | | 72 | 44 | 26 | 28 | 1 | 1 |
| 220 | | | 56 | 15 | 71 | 45 | 31 | 32 | 1 | 1 |
| 223 | | | | 71 | 71 | 50 | 31 | 32 | 1 | 2 |
| 224 | 86 | | | 8 | 94 | 35 | 7 | 14 | 0 | 0 |
| 227 | | 86 | | 8 | 94 | 39 | 15 | 21 | 0 | 0 |
| 230 | | | 86 | 8 | 94 | 45 | 29 | 31 | 1 | 1 |
| 233 | | | | 93 | 93 | 50 | 33 | 34 | 2 | 2 |
| 226 | 100 | | | | 100 | 34 | 4 | 9 | 0 | 0 |
| 225 | 86 | | | 15 | 101 | 36 | 12 | 20 | 0 | 0 |
| 229 | | 102 | | | 102 | 38 | 10 | 16 | 0 | 0 |
| 228 | | 86 | | 17 | 103 | 40 | 20 | 25 | 0 | 0 |
| 232 | | | 103 | | 103 | 44 | 27 | 29 | 1 | 1 |
| 231 | | | 86 | 15 | 101 | 45 | 28 | 31 | 1 | 1 |
| 234 | | | | 102 | 102 | 50 | 32 | 33 | 2 | 2 |
| 235 | | | | 103 | 103 | 50 | 31 | 33 | 2 | 3 |
| 236 | | | | 128 | 128 | 50 | 32 | 32 | 4 | 4 |

[a] Samples 102 *etc.* from Field 1, 202 *etc.* from Field 2.

[b] Periods in storage at different temperatures given from left to right as they occurred in the experiment.

[c] Cull net necrosis or stem-end browning is that which causes more than 5 per cent sliced-off waste.

[d] "T" means "trace" or less than 0.5 per cent.

results given in table 5 were used to determine correlation between the average storage temperature and the percentage of cull net necrosis and of total net necrosis. The correlation coefficients are respectively + 0.845 and + 0.852 for the samples from Field 1 and + 0.893 and + 0.879 for the samples from Field 2.[3] The relationship may be expressed by equations for linear regression as follows:

Field 1, cull net-necrosis percentage = 0.57 (average ° F.—32.7° F.)
Field 2, cull net-necrosis percentage = 1.73 (average ° F.—32.1° F.)
Field 3, cull net-necrosis percentage = 0.56 (average ° F.—29.0° F.)
Field 4, cull net-necrosis percentage = 1.47 (average ° F.—27.8° F.)

This means, for example, that for every degree of temperature over 32.7° F., the samples from Field 1 developed 0.57 per cent cull net necrosis. Therefore the proportion of tubers developing either cull net necrosis or all degrees of net necrosis was determined largely by the excess of average temperature over a given temperature that varied, with the four sets of determinations, from 27.8° to 32.7° F.

[3] Highly significant (18, Table 16).

TABLE 6.—*Rate of development of net necrosis and stem-end browning in crops of 1940 to 1945, in storage at or near optimum temperature*

| Crop year | Field and series | Date of harvest | Tubers with net necrosis | | | Tubers with stem-end browning | | | Tubers per sample |
|---|---|---|---|---|---|---|---|---|---|
| | | | At harvest | Maximum | Date of maximum | At harvest | Maximum | Date of maximum | |
| | | | *Per cent* | *Per cent* | | *Per cent* | *Per cent* | | |
| 1940 | 1–B | | | 59 | Apr. 25 | | 11 | Jan. 9 | Over 800 |
| | 1–C[a] | | | 33 | Dec. 12 | | 6 | Nov. 22 | Over 700 |
| | 2–B | | | 20 | Jan. 27 | | 7 | Dec. 19 | Over 800 |
| | 2–C | | | 15 | Mar. 4 | | 7 | Dec. 2 | Over 800 |
| | 3–B | | | 8 | Apr. 26 | | 20 | Jan. 28 | Over 900 |
| | 3–C | | | 8 | Mar. 5 | | 17 | Jan. 16 | Over 900 |
| | 4–B | | | T[b] | Apr. 29 | | 32 | Apr. 29 | Over 1000 |
| | 4–C | | | 1 | Oct. 21 | | 36 | Dec. 16 | Over 1000 |
| 1941 | 1–A | Sept. 22 | 7 | 42 | Dec. 23 | 2 | 10 | Jan. 22 | Over 600 |
| | 1–B | Sept. 22 | 6 | 41 | Nov. 26 | 2 | 13 | Dec. 27 | Over 600 |
| | 2–A | Sept. 30 | 0 | 1 | Dec. 30 | 4 | 39[c] | Jan. 30 | Over 600 |
| | 2–B | Sept. 30 | 0 | 1 | Jan. 2 | 5 | 53[c] | Nov. 18 | Over 600 |
| | 3–A | Sept. 29 | 4 | 31 | Feb. 4 | 0 | 5 | Feb. 4 | Over 500 |
| | 3–B | Sept. 29 | 4 | 24 | Feb. 6 | 0 | 13 | Feb. 6 | Over 500 |
| | 4–A | Oct. 6 | 10 | 34 | Nov. 18 | 1 | 6 | Mar. 10 | Over 600 |
| | 4–B | Oct. 6 | 10 | 38 | Nov. 19 | 1 | 11 | Feb. 17 | Over 600 |
| 1942 | 1 | Sept. 17 | 0 | T | Oct. 19 | 0 | 22 | Dec. 17 | About 700 |
| | 2 | Sept. 23 | 0 | 2 | Jan. 23 | T | 42 | Jan. 23 | About 800 |
| | 3 | Sept. 12 | 12 | 36 | Dec. 12 | 0 | 6 | Dec. 12 | About 500 |
| | 4 | Sept. 30 | T | 17 | Jan. 28 | 0 | 16 | Dec. 30 | About 700 |
| 1943 | 1 | Sept. 20 | 0 | 4 | Dec. 29 | 1 | 41 | May 16 | About 400 |
| | 2 | Oct. 13 | 0 | 1 | Dec. 2 | 1 | 2 | Feb. 10 | About 400 |
| | 3 | Sept. 25 | 3 | 26 | Dec. 14 | T | 10 | Oct. 15 | About 400 |
| | 4 | Oct. 4 | 2 | 20 | Jan. 4 | 1 | 13 | Oct. 4 | Over 500 |
| 1945 | 1 | Sept. 20 | T | 12 | Nov. 29 | 0 | 36 | Dec. 27 | About 600 |
| | 2 | Oct. 6 | 1 | 34 | Jan. 7 | 0 | 4 | Feb. 11 | About 700 |

[a] From part of field treated differently from other parts sampled.
[b] "T" means "trace" or less than 0.5 per cent.
[c] The maximum was 39 per cent at 47° F. and 53 per cent at 60° F.

There is not a similar relationship between temperature and stem-end browning in this test, because the lower temperatures had a greater inhibiting effect upon this kind of discoloration than upon net necrosis.

### EFFECT OF TIME OF EXAMINATION

All internal discoloration had developed by December 15 in the 1937 crop. In the 1938 crop, the maximum of net necrosis was reached by November 23 and December 27, respectively, in two series, but of stem-end browning not before February 11 (time of last examination of any samples). In the 1939 crop, the maximum net necrosis was reached in February and the maximum stem-end browning by mid-December. Similar data for the composite samples of later crops are given in table 6, and show that there usually was some net necrosis and stem-end browning at harvest. Harvest dates occurred from September 12 to October 13. Most of either kind of dis-

TABLE 7.—*Net necrosis and stem-end browning in duplicate samples in the same series*

| Crop | Temperature | Period | Field | Sample | Special treatment | Net necrosis | Stem-end browning |
|---|---|---|---|---|---|---|---|
| | *°F.* | *Days* | | | | *Per cent* | *Per cent* |
| 1942 | 52 | 150 | 1 | A | ..... | 0.1 | 19.6 |
| | | | | B | ..... | 0.6 | 19.7 |
| | | | 2 | A | ..... | 1.2 | 41.0 |
| | | | | B | ..... | 0.6 | 37.8 |
| | | | 3 | A | ..... | 35.5 | 6.2 |
| | | | | B | ..... | 36.1 | 6.1 |
| | | | 4 | A | ..... | 15.1 | 15.2 |
| | | | | B | ..... | 15.3 | 14.4 |
| 1943 | 50 | 120 | 1 | A | ..... | 0.3 | 24.6 |
| | | | | B | ..... | 1.1 | 30.7 |
| | | | | C | Clipping[a] | 1.8 | 4.3 |
| | | | | D | Splitting[b] | 1.9 | 5.3 |
| | | | | E | Do | 0.4 | 5.1 |
| | | | 2 | A | ..... | 0.3 | 2.0 |
| | | | | B | ..... | 0.5 | 2.3 |
| | | | | C | Clipping | 0.0 | 0.0 |
| | | | | D | Splitting | 0.0 | 0.0 |
| | | | | E | Do | 0.0 | 0.0 |
| | | | 3 | A | ..... | 20.9 | 4.9 |
| | | | | B | ..... | 20.5 | 4.7 |
| | | | | C | Clipping | 10.1 | 0.3 |
| | | | | D | Splitting | 6.1 | 0.0 |
| | | | | E | Do | 4.1 | 1.0 |
| | | | 4 | A | ..... | 19.6 | 8.4 |
| | | | | B | ..... | 18.1 | 8.0 |
| | | | | C | Clipping | 14.0 | 0.0 |
| | | | | D | Splitting | 10.4 | 0.0 |
| | | | | E | Do | 14.6 | 0.0 |
| 1945 | 50 | 100 | 1 | A | ..... | 9.8 | 35.8 |
| | | | | B | ..... | 10.1 | 34.1 |
| | | | 2 | A | ..... | 33.3 | 2.0 |
| | | | | B | ..... | 33.4 | 2.6 |

[a] Each tuber had ⅛ inch of the stem-end clipped off at the beginning of storage.
[b] Each tuber was split at the beginning of storage, half going into sample D and half into sample E.

coloration developed in storage. Net necrosis reached a maximum by October 19 to April 29, usually by the end of January. Stem-end browning reached a maximum by October 4 to May 16, usually by the end of February.

### DUPLICATES

Comparisons 2, 6, and 7 of table 1 show that, in the 1940 crop, duplicate samples in the same series developed practically the same percentages of tubers affected with net necrosis or stem-end browning if kept for the same length of time at the same temperature, even if in different lockers.

Duplicate samples of the crop of 1942 were kept at 52° F. for 150 days. The results of examination, given in table 7, show very close agreement between duplicates.

Duplicate samples of the crop of 1943 were kept at 50° F. for 120 days. The results, given in table 7, show very close agreement between nontreated duplicates in most cases. Duplicates with the stem-end clipped off for $\frac{1}{8}$ inch at the beginning of storage had net necrosis usually reduced and stem-end browning eliminated or greatly reduced. Splitting of the tubers at the beginning of storage to make two split-tuber duplicate samples had the same reducing effect as clipping, and the split-tuber duplicates often agreed with one another less than the whole-tuber duplicates in the same series.

Duplicate samples of the crop of 1945, kept at 50° F. for 100 days, also agreed closely one with another (Table 7).

Altogether, these results showed that the composite samples made as described were replicates very similar as to potentiality for the development of net necrosis or stem-end browning. Repeated examination of the same tubers apparently does not yield reliable data.

### EFFECT OF SIZE OF TUBER

Using the crop of 1937, Folsom and Rich (4, p. 321) showed that stem-end browning increased in frequency as tuber weight decreased, while net necrosis, where abundant, decreased in frequency.

When the 72 samples from the crop of 1945 were examined, in each instance the record of net necrosis and stem-end browning was made by centuries of tubers, always examining in turn the 100 apparently largest as a group until the sample was finished. From the records thus obtained, the average was determined for the 8 or 9 centuries of largest tubers stored entirely or predominantly at 34° F., then for the next largest tubers, and so on; this was repeated for temperatures of 38°, 44°, and 50° F.

The results, in table 8, show that in each field, at every storage temperature, the percentage of tubers developing net necrosis, whether cull, mild, or total, tended to decrease with smaller tuber size with few exceptions. For example, the part of the crop from Field 2 kept at 50° F. averaged 46.3 per cent net necrosis for the largest-tuber centuries and decreased, as relative tuber size decreased, to 41.5, 37.1, 33.0, 23.6, 21.4, and 17.9 per cent, respectively, for the centuries with progressively smaller tubers. One pos-

TABLE 8.—*Effect of tuber size on net necrosis and stem-end browning. Crop of 1945*

| Field | Temperature[a] | Century[b] | Tubers with net necrosis[c] | | | Tubers with stem-end browning | | | Ratio cull percentage to total percentage | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cull[d] | Mild[e] | All | Cull[d] | Mild[e] | All | N.N. | S.E.B. |
| | °F. | | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | | |
| 1[f] | 34 | 1 | 1.0 | 5.1 | 6.1 | 0 | 0 | 0 | 0.164 | ..... |
| | | 2 | 0.8 | 2.8 | 3.6 | 0 | 0 | 0 | 0.222 | ..... |
| | | 3 | 0.9 | 2.3 | 3.2 | 0 | 0 | 0 | 0.281 | ..... |
| | | 4 | 0.7 | 1.6 | 2.2 | 0 | 0 | 0 | 0.318 | ..... |
| | | 5 | 0.8 | 1.0 | 1.8 | 0 | 0 | 0 | 0.444 | ..... |
| | | 6 | 0.9 | 0.6 | 1.4 | 0 | 0 | 0 | 0.643 | ..... |
| | 38 | 1 | 4.2 | 7.0 | 11.2 | 0 | 0 | 0 | 0.375 | ..... |
| | | 2 | 3.2 | 3.6 | 6.8 | 0 | 0 | 0 | 0.471 | ..... |
| | | 3 | 3.2 | 3.0 | 6.2 | 0 | 0 | 0 | 0.516 | ..... |
| | | 4 | 3.2 | 1.8 | 5.0 | 0 | 0 | 0 | 0.640 | ..... |
| | | 5 | 2.7 | 1.2 | 3.9 | 0 | 0 | 0 | 0.692 | ..... |
| | | 6 | 1.8 | 1.1 | 2.9 | 0 | 0 | 0 | 0.621 | ..... |
| | 44 | 1 | 12.6 | 2.0 | 14.6 | 0 | 2.0 | 2.0 | 0.863 | 0.000 |
| | | 2 | 11.1 | 2.4 | 13.6 | 0.2 | 2.6 | 2.8 | 0.816 | 0.071 |
| | | 3 | 8.7 | 1.9 | 10.6 | 0.3 | 4.6 | 4.9 | 0.821 | 0.061 |
| | | 4 | 8.0 | 1.3 | 9.3 | 1.2 | 5.1 | 6.3 | 0.860 | 0.190 |
| | | 5 | 4.7 | 1.2 | 5.9 | 1.6 | 3.1 | 4.7 | 0.797 | 0.340 |
| | | 6 | 4.4 | 0.4 | 4.9 | 2.1 | 1.6 | 3.7 | 0.898 | 0.568 |
| | 50 | 1 | 16.6 | 1.9 | 18.5 | 1.3 | 21.3 | 22.5 | 0.897 | 0.058 |
| | | 2 | 12.0 | 1.1 | 13.1 | 3.8 | 23.5 | 27.3 | 0.916 | 0.139 |
| | | 3 | 9.4 | 0.8 | 10.1 | 10.0 | 18.3 | 28.3 | 0.931 | 0.353 |
| | | 4 | 9.9 | 1.6 | 11.5 | 13.9 | 14.5 | 28.4 | 0.861 | 0.489 |
| | | 5 | 6.1 | 1.8 | 7.9 | 16.5 | 8.1 | 24.6 | 0.772 | 0.671 |
| | | 6 | 4.9 | 1.4 | 6.3 | 15.5 | 3.3 | 18.8 | 0.778 | 0.824 |
| 2[g] | 34 | 1 | 7.4 | 14.7 | 22.1 | 0 | 0 | 0 | 0.335 | ..... |
| | | 2 | 6.6 | 9.2 | 15.8 | 0 | 0 | 0 | 0.418 | ..... |
| | | 3 | 6.9 | 7.0 | 13.9 | 0 | 0 | 0 | 0.496 | ..... |
| | | 4 | 5.3 | 4.1 | 9.5 | 0 | 0 | 0 | 0.558 | ..... |
| | | 5 | 4.3 | 2.1 | 6.4 | 0 | 0 | 0 | 0.672 | ..... |
| | | 6 | 5.1 | 0.9 | 6.0 | 0 | 0 | 0 | 0.850 | ..... |
| | | 7 | 3.1 | 0.2 | 3.3 | 0 | 0 | 0 | 0.939 | ..... |
| | 38 | 1 | 18.2 | 12.6 | 30.8 | 0 | 0 | 0 | 0.591 | ..... |
| | | 2 | 13.0 | 8.3 | 21.3 | 0 | 0 | 0 | 0.610 | ..... |
| | | 3 | 12.8 | 5.6 | 18.3 | 0 | 0 | 0 | 0.699 | ..... |
| | | 4 | 11.0 | 3.4 | 14.4 | 0 | 0 | 0 | 0.764 | ..... |
| | | 5 | 9.3 | 2.1 | 11.4 | 0 | 0 | 0 | 0.816 | ..... |
| | | 6 | 8.1 | 1.2 | 9.3 | 0 | 0 | 0 | 0.871 | ..... |
| | | 7 | 6.3 | 0.2 | 6.6 | 0 | 0 | 0 | 0.955 | ..... |
| | 44 | 1 | 37.4 | 5.0 | 42.4 | 0.1 | 0.1 | 0.2 | 0.882 | 0.500 |
| | | 2 | 33.3 | 4.1 | 37.4 | 0.1 | 0.1 | 0.2 | 0.890 | 0.500 |
| | | 3 | 25.6 | 3.4 | 29.0 | 0.6 | 0.1 | 0.7 | 0.883 | 0.857 |
| | | 4 | 25.8 | 1.4 | 27.2 | 0.6 | 0.1 | 0.7 | 0.949 | 0.857 |
| | | 5 | 21.7 | 1.2 | 22.9 | 0.9 | 0.2 | 1.1 | 0.948 | 0.818 |
| | | 6 | 19.3 | 0.8 | 20.1 | 1.0 | 0.1 | 1.1 | 0.960 | 0.909 |
| | | 7 | 16.0 | 0.2 | 16.2 | 0.8 | 0 | 0.8 | 0.988 | 1.000 |
| | 50 | 1 | 42.5 | 3.8 | 46.3 | 0.1 | 0.4 | 0.5 | 0.918 | 0.000 |
| | | 2 | 39.5 | 2.0 | 41.5 | 0.8 | 0.3 | 1.0 | 0.952 | 0.800 |
| | | 3 | 35.0 | 2.1 | 37.1 | 1.5 | 0.3 | 1.8 | 0.943 | 0.833 |
| | | 4 | 31.4 | 1.6 | 33.0 | 2.0 | 0.3 | 2.3 | 0.952 | 0.870 |
| | | 5 | 23.0 | 0.6 | 23.6 | 2.4 | 0.1 | 2.5 | 0.975 | 0.960 |
| | | 6 | 20.3 | 1.1 | 21.4 | 3.5 | 0.4 | 3.9 | 0.949 | 0.897 |
| | | 7 | 17.5 | 0.4 | 17.9 | 2.9 | 0 | 2.9 | 0.978 | 1.000 |

[a] Temperature at which all or most of the storage period of 35 to 128 days was spent.

[b] Each 100 tubers were selected as the largest appearing ones in the sample. Hence those in the first century were the largest, those in the second were the next largest, *etc.*

[c] Each figure represents 800 or 900 tubers, 100 from each of 8 or 9 samples.

[d] Cull net necrosis or stem-end browning is that which causes more than 5 per cent sliced-off waste.

[e] Mild net necrosis or stem-end browning is that which causes less than 5 per cent sliced-off waste.

[f] With seed-originated leafroll in 5 per cent of the hills.

[g] With seed-originated leafroll in 12 per cent of the hills.

sible explanation of this positive correlation between relative tuber size and percentage of tubers with net necrosis is that there was a corresponding correlation between relative tuber size and new leafroll infection. Another is that with the same incidence of leafroll infection there was a tendency for larger tubers to react more often than smaller ones by developing net necrosis. Finally, both may occur.

The results with respect to stem-end browning were somewhat similar in the 44° and 50° F. series of samples from Field 2, in cull and total percentages. Here the percentage tended to increase with smaller tuber size. In Field 1 there was no correlation between tuber size and total percentage. The cull percentage increased and the mild percentage decreased with smaller tuber size, presumably because stem-end browning was shallow in all tubers and caused 5 per cent waste more often in smaller tubers.

An increase in the ratio of cull percentage to total percentage with a decrease in tuber size, was true (Table 8, last two columns) generally of both kinds of discoloration. It was less pronounced with respect to net necrosis in samples held at 44° and 50° F., probably because these temperatures were optimum not only for percentage of tubers developing the symptom but also for penetration deep enough to cause 5 per cent waste even in most of the larger tubers.

### RATIO OF NET NECROSIS TO NEW LEAFROLL INFECTION

Net necrosis appears in late summer or fall before harvesting, or in storage. It appears only in tubers that have been invaded by the leafroll virus during the growing season in which they were produced. Such tubers may be called potentially net-necrosis tubers. The proportion of these that actually develop net necrosis is a ratio that sometimes varies with tuber size.

Using data of Schultz and Folsom (16, p. 70) on the crop of 1919, the following relation between tuber weight and percentage of newly infected leafroll tubers showing net necrosis is calculable for three seed stocks:

| Tuber weight, in oz. | | 2 | 2–3 | 2–4 | 2–5 | 2–6 | 2–7 | 2–8 | 2–9 | 2–10 | 2–11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NN/LR, in per cent, lot | A | 54 | 59 | 65 | 66 | 67 | 68 | 69 | 71 | 72 | |
| | B | 67 | 67 | 75 | 71 | 75 | 72 | 72 | 72 | 72 | 67 |
| | C | 0 | 100 | 100 | 83 | 82 | 88 | 87 | 87 | 87 | |

In lot A there is a consistent increase in the ratio as the average tuber weight is greater. The same relation is evident from further data of Schultz and Folsom (17, p. 64) on the crop of 1921:

| Tuber weight, in oz. | 1–2 | 1–3 | 1–4 | 1–5 | 1–6 | 1–7 | 1–8 |
|---|---|---|---|---|---|---|---|
| NN/LR, in per cent | 45 | 59 | 64 | 67 | 71 | 76 | 79 |

The proportion of net necrosis to new leafroll infection probably varies also with variety, temperature, length of time elapsed after maturing of the tubers, time of plant inoculation by aphids,[4] time of harvesting (11, p. 296),

[4] The results of grafting by Bonde, Simpson, and Hovey (15) in the summer of 1944 were that the proportion of leafroll tubers that showed net necrosis rose from 0.08 in grafts made July 5 to 0.82 in grafts made August 9 and then declined to 0.53 in grafts made September 2. If experimental infection by aphids should show a similar correlation between time of infection and proportion of newly infected leafroll tubers that get net necrosis, it would be reasonable to suppose that the proportion referred to might vary from one field to another according to the time of the greatest infection by aphids.

degree of maturity at harvesting (10, p. 498), and kind and amount of mineral fertilizer applied (11, p. 297–298; 12, p. 432–433; 13, p. 218–219). It therefore should not be surprising to find that the NN/LR ratio varies considerably even when all the leafroll is of recent infection. Folsom and Rich (4, p. 315) found in the crop of 1937 an average ratio of about 1:3.3, with extremes of 1:1 and 1:9, among 29 Green Mountain seed stocks. In the 1939 crop grown on Highmoor Farm, near Monmouth in southwestern Maine, although half the tubers were newly infected with leafroll there was no net necrosis in samples stored at 32°, 37°, 41°, 45°, 54°, 72°, 77°, and 86° F.

In Aroostook-grown samples of the crop of 1942, the ratio of net necrosis percentage (at 51° F.) to new leafroll percentage varied considerably between different varieties and between different samples of Green Mountains. It was less than 0.01 for Chippewa, about 0.05 for Sequoia, about 0.10 for Katahdin, about 0.20 for Sebago, from about 0.20 to 0.40 for Irish Cobbler, and 0.27 to 1.00 (averaging 0.79) for 14 Green Mountain samples, as compared with 0.20 for Green Mountain grown on Highmoor Farm (12, p. 220 and 276).

### EFFECT OF REGION

Stem-end browning was found in 28 of 30 bins examined in central Maine in the 1929 crop, with an average of 9 per cent of the tubers affected. The 1929 crop in Aroostook County in northeastern Maine had considerable net necrosis, but only one stored stock had an appreciable amount of stem-end browning. The 1937 crop in Aroostook County had still more net necrosis, but of 52 stored stocks examined, only 4 had more than 1 per cent of the tubers with stem-end browning.

At harvest time in 1942 in Aroostook County, soil temperature was found to be slightly below the optimum temperature for the development of net necrosis. One locker was held at the same temperature as that of the soil during harvest time, and samples kept in it developed almost as much net necrosis and stem-end browning as samples kept at 51° F.

In Pennsylvania[5] although several types of stem-end browning were troublesome, leafroll net necrosis was seldom found.

In New York, on Long Island, Green Mountains are subject to considerable leafroll spread, but develop little if any net necrosis. The reason probably is the high soil temperature prevailing (over 70° F.) up to the time potatoes are harvested (1, p. 9).

Aphid transmission of leafroll to caged plants in Wisconsin in 1927 induced net necrosis evident on October 25, two months after harvesting, in 50 to 100 per cent of the tubers (6, p. 28–31). Net necrosis apparently was common in Vermont in 1926 (6, p. 10).

Considerable spread of leafroll occurred in Indiana in about 1922 but apparently without causing net necrosis (5) although the variety concerned has shown net necrosis in Maine.

[5] Letter of April 25, 1940, from K. W. Lauer, Plant Pathologist of the Pennsylvania State Dept. of Agric., in charge of potato seed certification.

Generally it seems from the literature and from the temperature relations disclosed in this paper, that net necrosis can develop as a symptom of leafroll only in certain northern states. If a state or potato region is far enough South, soil, storage, and market are too warm for the development of net necrosis even if leafroll spreads readily and varieties are grown that easily develop the symptom at optimum temperatures. If a state or potato region is far enough North, leafroll is no common and does not spread often. There is a zone where leafroll spreads fairly often and where the temperature at the end of the growing season and at the beginning of the storage season favors the development of net necrosis; here either potato growers learn to grow a variety with practically no net-necrosis potentiality (such as Bliss Triumph, Katahdin, or Chippewa) or else take their losses from growing varieties (such as Green Mountain) that will develop the symptom when leafroll dissemination, optimum storage temperatures, and other factors favorable to the expression of this defect occur in the same season. After working in Maine storages (2), Edgar writes (letter of September 18, 1945) that "in the early part of storage the average temperatures in Maine storages will be 48°; the average to January 1 is usually 44° although the temperature January 1 may be below 40°." Therefore most storage temperatures in Maine may be considered generally about optimum for the development of net necrosis in Green Mountains under the prevailing conditions of well-distributed Chippewa and Green Mountain sources of leafroll dissemination.

### INCREASE IN TRANSIT

Eight stocks of potatoes of the crop of 1939 were sampled by Cedric Porter, inspector for the Division of Markets, Maine State Department of Agriculture. From each stock three similar samples were taken. One was examined immediately, one was returned to the storage whence it came, and one was sent on with a carload of potatoes and was examined by one or another local inspector at the point of destination of the shipment (Washington, D. C., Pittsburgh, Cleveland, Baltimore, Philadelphia, Brooklyn, or Boston). The percentage of potatoes commercially defective[6] from internal discoloration of the net-necrosis and stem-end browning types increased more in home storage than in transit in four out of six comparisons where there was an increase. In the other two comparisons it decreased less in one case. Therefore there was no evidence that shipment induced consistently either more or less increase than home storage.

However, in the case of stocks kept at minimum temperatures, there could be an increase of net necrosis and stem-end browning in transit and

[6] "Commercially defective" means that a tuber loses over 5 per cent of its weight in the course of having all discolored tissue sliced off. The 5 per cent waste test disqualifies some small tubers because of stem-end browning, in spite of the discoloration being comparatively shallow. Since waste is determined by slices at any angle to give elimination of internal discoloration with the least loss of weight, some large tubers may have net necrosis without being disqualified by the 5 per cent test. Inspectors do not differentiate the two kinds of discoloration in their records and do not include all that there is of either kind in their records.

market, especially early in the season, due to change of temperature from too low to optimum. After low temperature has acted long enough on a stock to permanently inhibit further development of these internal discolorations even at optimum temperature, shipments from such stock may be characterized by much less internal discoloration than parallel shipments from other stocks not kept at a low enough temperature to prevent the development of internal discoloration within 90 to 120 days after harvest.

The results previously described for the samples of the crop of 1945 show that considerable increase in percentage of tubers with net necrosis can occur in a week or two at 50° F. following periods at lower temperatures. This could easily explain some differences between inspectors' reports at point of shipment and those at destination. However, such differences could be due in part to unreliable or insufficient sampling, bearing in mind especially the effect of tuber size.

## CONTROL

Control of net necrosis or stem-end browning through the permanently inhibiting effect of an initial period at a low temperature probably would require artificial refrigeration of storages. The low temperatures required would prohibit keeping Chippewa or Katahdin potatoes in the same storage because of mahogany browning (7; 12, p. 291). Control of stem-end browning through the permanently inhibiting effect of an initial period at a high temperature such as 70° would require heating of storages within a very narrow range, inasmuch as 60° F. is not far from the optimum for development of the discoloration. Heat prevention might be practicable for the control of net necrosis. However, heating may be dangerous inasmuch as the percentage of tubers developing late blight has been increased from less than 5 per cent, at 32–36° F. for 60 days followed by 50° F. for 60 days, to over 20 per cent, at 65–70° F. for 15 to 60 days followed by 50° for 60 days (13, p. 288).

Knowledge of the optimum temperature and required period of time for the development of net necrosis and stem-end browning has helped indirectly toward control through improving tests of the effects of strain, variety, fertilizer, *etc.*, upon these discolorations.

The work reported in this paper indicates that the control of leafroll through selection of stocks showing little or no net necrosis in samples kept at optimum temperature, is not feasible because of the low ratio of net necrosis to new leafroll infection in some fields.

Reasonably good control of stem-end browning in the Green Mountain variety is possible through the use of seed stocks tracing back to certain sources in Vermont, Minnesota, and southwestern Maine (14, p. 530–531). An example was noted in discussing the temperature experiments with the crop of 1943.

The most desirable means for the control of net necrosis and stem-end browning probably would be commercially suitable new varieties immune from the causes of both.

## DISCUSSION

The fact that early storage temperatures may determine largely the amount of net necrosis or stem-end browning, explains in part how differences can arise with respect to these forms of internal tuber discoloration in different storage houses or in different bins in the same storage house even when filled from the same field. Differences in elevation of storage bins, in ventilation practices, in insulation, or in the temperature of the tubers harvested at different times, can influence the temperature enough to affect the development of the maladies in question. Interseasonal differences also may be explained in part by differences in temperature at the time of maturity, harvest, and fall storage.

Interesting questions awaiting biochemical study are why storage temperature influences the development of net necrosis and stem-end browning, why clipping of the stem-end and tuber splitting reduce such development, why tuber size apparently sometimes influences this development, why leafroll infection induces net necrosis only when of recent occurrence, and why only part of such recent leafroll infection induces net necrosis even at optimum temperature.

## SUMMARY

Leafroll net necrosis of potato tubers develops in Maine mostly during the first two or three months after harvesting. The percentage of incidence becomes highest at constant temperatures of about 45° to 50° F. and is very low at 33° and 70°. These threshold temperatures in 60 days can change potentially net-necrosis tubers so that they will not develop the discoloration later even upon being subjected to optimum temperatures. Greater tuber size is sometimes correlated with greater frequency of net necrosis and with a higher proportion of net-necrosis tubers among those recently invaded by the leafroll virus. Varieties that do not develop net necrosis as a leafroll symptom become relatively more desirable when the spread of leafroll increases in a region characterized by soil and storage temperatures that are about optimum for net necrosis. Net necrosis can sometimes increase considerably in transit and market in the event of a change in temperature toward the optimum. Various factors can influence storage temperature and so indirectly affect net necrosis. A number of field factors, but not relative humidity in storage, also can affect net necrosis.

Stem-end browning of potato tubers in Maine, not attributable to bacterial or fungus organisms, but apparently requiring a virus for its development, reacts to temperature and some other factors much as does net necrosis. However, the critical temperatures are somewhat higher, and smaller tuber size rather than larger increases the percentage of incidence.

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# CONTROL OF VIRUS DISEASES OF CABBAGE SEED PLANTS IN WESTERN WASHINGTON BY PLANT BED ISOLATION

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## INTRODUCTION

Prior to 1942 practically all of the cabbage seed produced in the United States was grown in the Puget Sound area, the greatest concentration of acreage being in Skagit County, Washington. The lower Skagit valley is relatively small, being less than 25 miles in either dimension. Since cabbage is a biennial crop, there is no crucifer-free period during which disease inoculum and insect pests can be completely eliminated as a source of virus inoculum. Seed is sown in large plant beds[2] in early June and the ensuing seed crop harvested during July and August of the following year. Thus there is an overlapping period of about two months during which seedling plants for a crop just started are exposed to certain diseases and insect pests affecting the seed crops approaching harvest.

It is a regular occurrence in this area for infestations of the cabbage aphid (*Brevicoryne brassicae* L.) to build up in seed fields during spring and to reach peak intensity during this two-month overlapping period. Winged forms migrate thence into young plant beds when the latter are in close proximity. Since the cabbage aphid is a vector of crucifer viruses,[3, 4, 5] any such infection of seed plants would be readily transmitted to seedling plants. Such has actually been the case. In the seed crops harvested in 1943 and 1944 approximately 60 per cent of all seed plants were infected with cabbage mosaic.[6] Of 6 large plant beds sown in June, 1943, for the 1944 crop, 2 were immediately adjacent to such seed fields and the other 4 were less than 100 rods removed. By actual counts in 2 of these large plant beds, approximately 80 per cent of the plants were infected with mosaic before they were transplanted. During the same season a small experimental plant bed grown by the Seed Laboratory approximately 30 miles from the seed-growing area had no evidence of virus infection.

[1] Associate Pathologist, Division of Fruit & Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, in cooperation with the Washington Agricultural Experiment Stations, The Vegetable Seed Production Laboratory. Published as Scientific Paper No. 663, College of Agriculture and Agricultural Experiment Stations, State College of Washington, Pullman.

[2] The common practice is for a seed-contracting company to grow plants for its entire acreage in one large plant bed. These plants are distributed to growers at transplanting time.

[3] Larson, R. H., and J. C. Walker. A mosaic disease of cabbage. Jour. Agr. Res. [U.S.] **59**: 367–392. 1939.

[4] Tompkins, C. M. A transmissible mosaic disease of cauliflower. Jour. Agr. Res. [U.S.] **55**: 33–46. 1937.

[5] Tompkins, C. M., M. W. Gardner, and H. Rex Thomas. Black Ring, A virus disease of cabbage and other crucifers. Jour. Agr. Res. [U.S.] **57**: 929–943. 1938.

[6] Walker, J. C., Francis LeBeau, and Glenn S. Pound. Viruses associated with cabbage mosaic. Jour. Agr. Res. [U.S.] **70**: 379–404. 1945.

Beginning with the 1944–45 crop, it was recommended that seed companies isolate their plant beds, by several miles if possible, from such diseased fields. Consequently, 60 per cent of the plants transplanted in the fall of 1944 had been grown several miles from seed fields. The remaining 40 per cent of the plants were from plant beds located within the seed-growing area and near seed fields. For the 1945–46 crop over 90 per cent of the plants were from plant beds grown in isolated areas. It was thought that if healthy plants were used in the fall transplanting, the amount of mosaic in the seed fields would be materially reduced. Although it was surmised that aphid activity in the fall after transplanting would result in some infection, it was expected that the ultimate extent of infection would not be great. This hope was based on the failure to find turnip fields severely affected with mosaic. Turnip, which is very susceptible to mosaic, is sown in late fall after aphid activity is greatly reduced and thereby evidently escapes severe infections. This paper reports the effect of isolation of plant beds in reducing the amount of mosaic in cabbage seed fields.

## METHODS

About 6 to 8 weeks after transplanting, a systematic disease survey was made of the cabbage acreage in both 1944 and 1945. At least 70 per cent of the acreage planted from each plant bed was included in the survey. In 1944 a total of 85 fields, comprising 957 acres, was checked. This represented almost 80 per cent of the entire planting in the Skagit area. In 1945 only 63 fields and 601 acres were surveyed. This was approximately 72 per cent of the entire acreage. The number of virus-infected plants was counted in three sample rows (one on each side and one in the middle of the field) of 200 plants each for each field visited. If a field was planted with plants from different sources, 600 plants from each source were checked and the ultimate percentage infection of the field determined by averaging the values, weighting them according to the proportion of the field planted from each source.

In order to determine the extent of infection after the fall survey, the acreage of 1944–45 crop was again surveyed in the late spring of 1945 when plants were in early bloom, using the method described. This survey included 55 fields and 720 acres. Another limited survey was made one month prior to harvest in which a few selected fields were checked. This last survey was to determine the extent of severity of infections occurring in late spring.

## RESULTS

*The 1944–45 Crop.*—During the fall survey very great differences existed between plants from isolated plant beds and those from local, nonisolated plant beds. Plants from the isolated areas established themselves more quickly and were more vigorous when they entered the winter. Much of this difference in growth and vigor is attributed to the stunting of local-grown plants by the mosaic disease and to the relative freedom from this disease of plants from isolated areas.

Isolation of plant beds greatly reduced the amount of mosaic in transplanted fields in the fall (Table 1). Over 53 per cent of the plants from

TABLE 1.—*Effect of plant bed isolation on the amount of mosaic in cabbage seed fields, 1944–45*

| Source of plants and season of survey | Percentage of plants infected | Percentage of fields with: No infection | Over 15 per cent infection | Over 40 per cent infection | Over 60 per cent infection |
|---|---|---|---|---|---|
| Fields planted from isolated beds: Fall, 1944 | 0.8 | 42.0 | 0.0 | 0.0 | 0.0 |
| Fields planted from isolated beds: Spring, 1945 | 3.0 | 2.9 | 2.9 | 0.0 | 0.0 |
| Fields planted from local beds:[a] Fall, 1944 | 53.0 | 0.0 | 100.0 | 69.4 | 30.6 |
| Fields planted from local beds:[b] Spring, 1945 | 55.1 | 0.0 | 100.0 | 84.2 | 42.2 |

[a] Includes 2 large plant beds and several smaller ones. All were grown within 1 mile of a diseased seed field.

[b] Includes only the 2 large plant beds referred to in footnote a.

local plant beds had mosaic symptoms and no field in which they were used was free of infection. Only 0.8 per cent of the plants from isolated plant beds had mosaic symptoms, and 42 per cent of the fields had no infection at all. These results are in spite of the fact that aphids occurred in plant beds in sufficient amount to cause widespread infection had they migrated from virus-infected plants. Table 2 shows the average percentage infection

TABLE 2.—*Relation of plant bed isolation to amount of mosaic in cabbage seed fields, 1944–45*

| Plant bed | Degree of isolation from diseased seed fields | Average percentage of infected plants in transplanted fields |
|---|---|---|
| A | Excellent | 0.23 |
| B | Excellent | 0.27 |
| C | Excellent | 0.49 |
| D | Excellent | 1.00 |
| E | Poor | 46.90 |
| F | Very poor | 72.60 |

of the acreage planted from individual plant beds. Beds A, B, C, and D were in well-isolated areas, the closest (C) being 7 miles from the nearest seed field, with dense woods separating them. The other 3 were 20 miles or more from seed fields. Bed E was in the seed-producing area but was about 200 rods from the nearest seed field. Bed F was also within the seed-producing area and was only 20 rods from diseased seed fields. It is believed that infection found in fields using plants from isolated areas was initiated for the most part after transplanting, since many fields from each of the beds had no infection. These data show that isolation of plant beds was sufficient to provide healthy plants at transplanting time.

Since aphids were found in many of the transplanted fields it was necessary to check the acreage again in late spring in order to determine the extent of infection after the fall survey. This survey was made in early May when plants were in early bloom. Since aphid infestations in this area are very light in early spring and do not reach severe proportions until late May or June, it was believed that such a survey would reveal the approximate extent of losses this disease would effect in seed yields. The incubation period of the viruses in seed plants is 30 days or more and infections initiated as late as June would not effect serious losses.

This late spring survey (Table 1) revealed that the acreage planted from isolated plant beds was still markedly free of mosaic when compared with that from local plant beds, the average infections being 3.0 and 55.1 per cent, respectively. There was an increase in the amount of mosaic of both acreages of slightly over 2 per cent. The highest infection in any field from isolated plant beds was 20 per cent. Without exception, the fields showing the highest increase were those of late-maturing varieties which were transplanted earliest in the fall and thus exposed longer to viruliferous aphids. The increase in the varieties transplanted later in the fall was much less. There was also an apparent relation between the percentage increase of mosaic and the amount of aphids present in the fields in the fall.

Some fields provided excellent tests of whether the beneficial effects of plant bed isolation would be lost by aphid activity after transplanting. A few fields were set with plants from local plant beds except for 12 to 15 rows in the center of the field which were set with plants from the same seed source but grown in isolated areas. These plants were all of the same age and were transplanted (at midseason) and grown under identical conditions. In the fall, 70 per cent of the local-grown plants were infected with mosaic, while less than 1 per cent of the plants from isolated plant beds were diseased. At the time of the spring survey the plants from isolated plant beds had only 4 per cent and 1 per cent mosaic in 2 such fields and were markedly superior in growth and vigor. The yield of seed harvested from the plants from the isolated plant bed in one of the fields was 220 per cent that harvested from a comparable sample from plants grown locally.

The average yield of seed in 1944–45 on 582 acres grown from plants from isolated plant beds was 628 pounds per acre. In the same season the average yield from 485 acres grown from plants from local plant beds was 359 pounds per acre. This constitutes a difference in average yields of 269 pounds per acre.

*The 1945–46 Crop.*—All of the plants for the 1945–46 crop were grown in well-isolated areas except for 6 small plant beds which provided plants for 100 acres. Four of these small beds were fairly well isolated while 2 were close to heavily infected fields. When this crop was surveyed in October, 1945, cabbage mosaic had been reduced to an almost insignificant status. The average percentage infection of the entire acreage was 2.4 per cent, and for the acreage planted from the local plant beds it was 10.1 per

cent. Not a single field planted from the isolated plant beds had as much as 10 per cent diseased plants; 36 per cent of the fields were free of infection and the average infection in this acreage was 0.9 per cent. Results obtained the previous season indicated that the percentage of infection would remain low until harvest, at least late enough in the second season to avoid serious yield reductions.

## DISCUSSION AND SUMMARY

The production of seed of biennial vegetable crops usually entails a system of culture that allows no crop-free period during which disease inoculum and insect pests can be destroyed or reduced. Under such conditions strict sanitation is necessary if a build-up of virus inoculum is to be avoided. Low standards of sanitation under such a system of culture created a tremendous build-up of virus inoculum in the cabbage seed crops of the Puget Sound area. Over 60 per cent of the plants of the entire cabbage seed acreage for 1943 and 1944 harvests were infected with cabbage mosaic. Severe reductions in seed yields resulted. It is difficult to control the cabbage aphid in this area sufficiently to prevent widespread virus infection. Consequently, it was necessary to avoid infection during the period when viruliferous aphids were extremely migratory. Infection of cabbage seed plants was avoided by isolation of plant beds from diseased seed fields.

In seed fields of the 1944–45 crop planted from isolated plant beds, the amount of mosaic 7 to 8 months after transplanting was only 5 per cent of that found in the crop, as a whole, grown in the same area during the 2 preceding years. This reduction is similar in degree when comparison is made with the 1945–46 fields planted from plant beds grown proximal to diseased seed fields. In the 1945–46 crop over 90 per cent of the plants were grown in well-isolated areas and the average infection of the entire acreage 8 weeks after transplanting was only 2.4 per cent. Thus, by plant bed isolation, cabbage mosaic has been reduced to a relatively insignificant status. It is thought that this reduction can be maintained by reasonable care and caution in locating plant beds. Wild cruciferous and non-cruciferous hosts do not appear, as a general rule, to be responsible for more than scattered infections in cabbage fields.

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# YIELD REDUCTIONS BY LOOSE SMUT OF WHEAT[1]

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The persistence of high levels of infection with loose smut, *Ustilago tritici* (Pers.) Rostr., in the soft red winter wheats of the middle western states during the past decade has frequently raised the question of the relation of the degree of infection to yield losses. This paper reports the results of a study of this question during 1941 and 1942. A study of the effects of loose smut on yield of barley has been published by Semeniuk and Ross (2) and the effects of simulated loose smut losses in wheat by Brown (1).

## METHODS AND MATERIALS

Two highly susceptible varieties, Purdue No. 1 and Wabash, were studied in 1941 and 1942. Two lots of seed of each variety from lightly and severely infected fields were obtained from the 1940 crop on the Purdue Soils and Crops farm at Lafayette, Indiana, for use in the 1941 study. The heavily infected seed of Purdue No. 1, studied in 1942, was obtained from a severely infected commercial field at Thorntown, Indiana, and the two lots of Wabash seed and the lightly infected Purdue No. 1 from the Purdue Soils and Crops farm. There was no evidence in the experimental plots of any significant amount of seed-transmitted disease other than loose smut.

In the 1941 studies, all seed was sown at the rate of 6 pecks per acre. In 1942, all Wabash seed and the lightly infected Purdue No. 1 was sown at the 6-peck rate. However, because of low percentage germination, the heavily infected Purdue No. 1 was sown at 9 pecks per acre. All seed was treated with one-half ounce per bushel of New Improved Ceresan (ethyl mercury phosphate). The number of heads of each variety (Table 1) in the severely smut-infected plots nearly equalled or exceeded those in the lightly infected plots.

The tests in both 1941 and 1942 were sown in randomized blocks with 10 replications. Each plot consisted of three 18-foot rows from which 16 feet of the center row was harvested for yield determinations. Counts of the numbers of heads produced and numbers of heads smutted were made on 3 feet of one end of a single row of each variety in each replication, after the row ends had been trimmed. The significance of differences was determined by the analysis of variance.

The theoretical yield of each variety on a smut-free basis was derived from the yield of the lightly infected entry corrected for an assumed yield reduction equal to the small percentage of loose smut infection. The yield reduction in a given variety attributable to the severe loose smut infection was calculated as the difference between the yield of these entries and the

[1] Cooperative investigations by the United States Department of Agriculture and the Purdue University Agricultural Experiment Station. (Journal Paper No. 244 of the Purdue University Agricultural Experiment Station.)

TABLE 1.—*Yields of 2 varieties of wheat, lightly or severely infected with loose smut, in 1941 and 1942*

| Wheat variety and data obtained | 1941 | | 1942[d] | |
|---|---|---|---|---|
| | Seed lightly infected | Seed severely infected | Seed lightly infected | Seed severely infected |
| Purdue No. 1 | | | | |
| Seeding rate per acre (pecks) | 6 | 6 | 6 | 9 |
| Head counts | | | | |
| Number[a] | 119.8 | 120.0 | 75.2 | 89.5 |
| Percentage smutted | 1.3 | 7.3 | 4.5 | 35.9 |
| Actual acre yield[b] (bu.) | 41.8 | 39.2 | 15.6 | 10.1 |
| Yield reduction[c] (per cent) | ..... | 7.4 | ..... | 38.2 |
| Wabash | | | | |
| Seeding rate per acre (pecks) | 6 | 6 | 6 | 6 |
| Head counts | | | | |
| Number[a] | 115.7 | 107.4 | 78.7 | 88.1 |
| Percentage smutted | 1.6 | 13.2 | 1.9 | 8.4 |
| Actual acre yield[b] (bu.) | 38.3 | 33.7 | 17.8 | 16.7 |
| Yield reduction[c] (per cent) | ..... | 13.4 | ..... | 7.9 |

[a] Means of 10 replications, each taken for 3 feet of drill row. Significant differences at the 5 per cent level between total numbers for different treatments = 10.7 heads in 1941 and 10.5 heads in 1942.

[b] Means of 10 replications. Significant yield differences between treatments at the 5 per cent level = 2.6 bushels in 1941 and 2.8 bushels in 1942.

[c] Percentage of the theoretical yield on a smut-free basis.

[d] Severe infestation with Hessian fly accounts for low yields.

theoretical yield on the smut-free basis of the variety concerned. These differences (Yield reductions in table 1) were expressed as the percentages of the theoretical yields.

## PRESENTATION OF DATA

In the 1941 tests, the numbers of heads per unit area of plot of the lightly and severely infected entries of each variety were closely comparable, the difference being considerably less than that required for significance. The percentage yield reductions attributable to the severe loose smut infection in both Purdue No. 1 and Wabash closely approximated the percentage of heads destroyed by loose smut. These comparisons involved a 7.4 per cent yield reduction in Purdue No. 1 associated with 7.3 per cent loose smut, and a 13.4 per cent yield reduction in Wabash associated with 13.2 per cent loose smut. The data are presented in table 1. The yield losses were significant at the 5 per cent level.

The results of the 1942 tests corresponded rather closely with those of the preceding year for the variety Wabash (Table 1). A much more severely infected seed lot of Purdue No. 1 was used in 1942. The smut infection in plots from this seed averaged 35.9 per cent and was attended by a yield reduction of 38.2 per cent. This seed, sown at a 9-peck rate, gave numbers of heads per unit area significantly greater than those from the lightly infected seed sown at the 6-peck rate.

## DISCUSSION AND CONCLUSIONS

The yield losses of grain, attributable to loose smut in the two wheat varieties studied, closely approximated the percentage of heads infected with loose smut in both 1941 and 1942.

The possibility of confusion in the interpretation of these results because of the possible occurrence of other seed-borne diseases in certain seed lots is not believed to be a significant consideration in this study. In the 1941 study all lots of seeds used were produced on the same farm. No significant infection with any seed-borne disease other than loose smut was to be observed in the plots in either year. Low germination in one seed lot, compensated for by increased seeding rate, was associated with a high proportion of thresher-injured and split kernels.

These results are in agreement with the conclusion of Brown (1) who deduced from experiments on head removal that reductions in grain yield of wheat, resulting from loose smut, would approximate the percentage of loose smut infection. The results are also similar to those of Semeniuk and Ross (2) who, working in Canada and Minnesota, found the percentage yield losses in spring barley to be approximately as great as the percentage of loose smut infection.

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# GLOEOSPORIUM VENETUM AND G. NECATOR: TWO DISTINCT SPECIES ON RUBUS[1]

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(Accepted for publication September 20, 1946)

An unfortunate assumption that *Gloeosporium necator* Ell. & Ev. was merely another name for *G. venetum* Speg. has resulted in a long-standing confusion in pathological literature relative to anthracnose of brambles (*Rubus*). The present discussion attempts to elucidate the several identities of the two species concerned in the light of the original applications of these names and of subsequent usage.

## GLOEOSPORIUM VENETUM SPEG.

The original diagnosis of *Gloeosporium venetum* Speg., published in 1879 (19, p. 477) is as follows:

> "*Gloeosporium* (Eugloeosp.) *Venetum* Speg. Maculae marginales, amorphae, maximae, vel centrales rotundae parvulae, mellae vel ochraceae linea fusco-purpurascente cinctae. Acervuli minuti, prominuli, gregarii vel solitarii, atri. Conidia ellipto-cylindracea, 7–8 = 2–2½, granulosa et guttalata, hyalina.
>
> "Hab. Ad folia viva vel languida, Rubi Chamaemori a Belluno & a Conegliano, Aut. 1877–78."

The material of this fungus available to us consists of a fragment obtained by Shear[2] from the specimen collected by Professor Spegazzini at Belluno, Italy, in October, 1878, and designated as No. 13 of his *Supplemento ad Decades Mycologicae italicae* preserved in the DeNotaris Herbarium at Rome.[3] We may consider the fragment as part of the type; it is but a single leaflet (Fig. 1, A and B) bearing fructifications and having the purple-bordered discoloration mentioned in the description.

On the basis of the leaflet at hand we have ascertained that the host of *Gloeosporium venetum* is definitely not *Rubus chamaemorus* L., cloudberry. Instead, it probably is one of the forms of *R. fruticosus* L., the common European species of blackberry.[4] It seems plausible to assume that Spegaz-

[1] An abstract of this paper for presentation has been published (13).

[2] In 1912 Dr. Shear, as pathologist in charge of diseases of small fruits in the Bureau of Plant Industry, U. S. Department of Agriculture, visited European mycological herbaria and was given fragments of typical material of fungi on these hosts, including that of *Gloeosporium venetum*.

[3] Lindau and Sydow (14, p. 557–559) and Trotter (21, p. 249–257) list *Decades Mycologicae italicae*, issued in 1879, among the publications of Spegazzini; however, we have found no reference to the supplement, nor do we know whether it was distributed to all the original subscribers. It is not with the set in the Division of Mycology and Disease Survey, which is a comparatively recent acquisition. The supplement is also absent from Spegazzini's own set of his *Decades Mycologicae italicae* in the Spegazzini Herbarium in the Instituto Botanica "Spegazzini," Museo, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina. We are indebted to Drs. L. R. Parodi and J. C. Linquist for this information supplied in 1943, together with the fact that there is entirely lacking in Spegazzini's herbarium any reference to the supplement, any specimens from it, or any authentic material of his *Gloeosporium venetum*.

[4] For this identification we are indebted to Dr. S. F. Blake, U. S. Dept. of Agriculture.

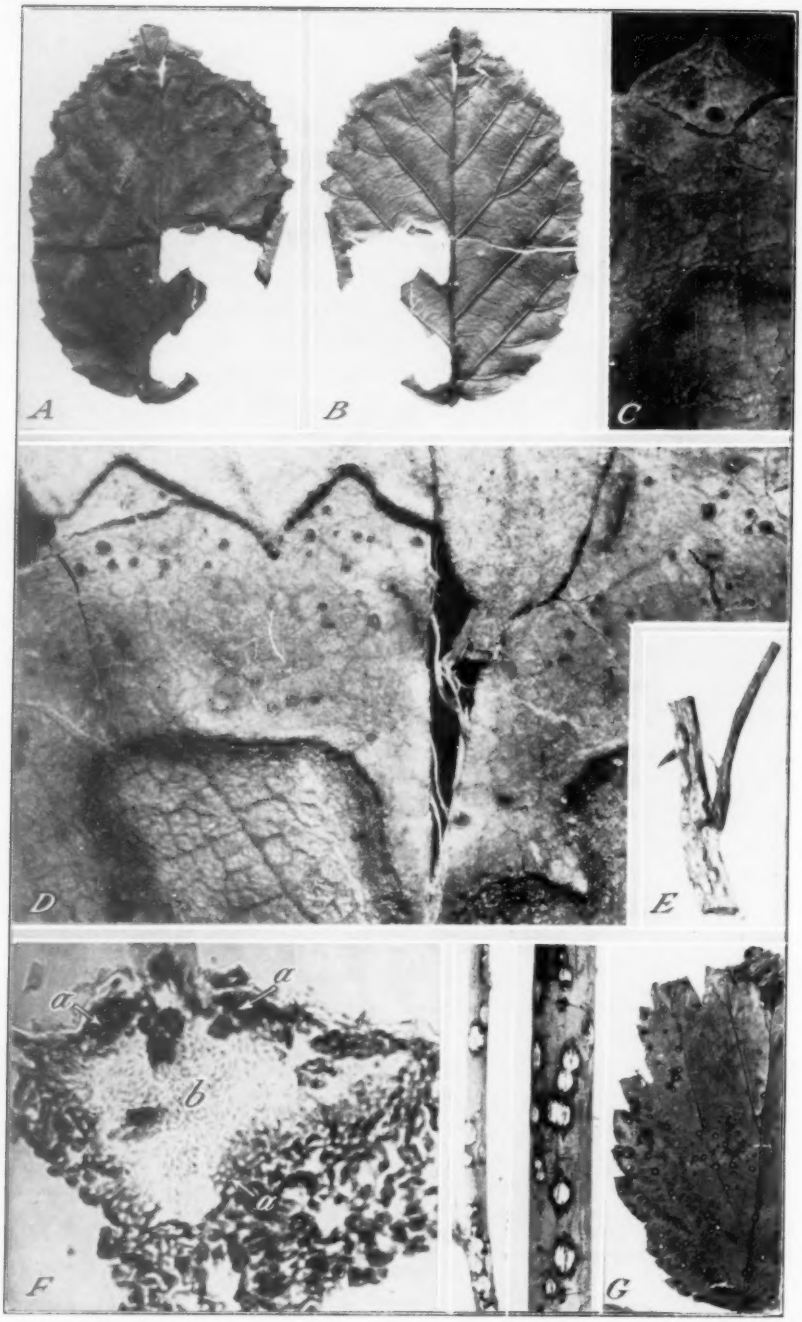


FIG. 1. A–D. *Glocosporium venetum* Speg. on leaflet of *Rubus*, presumably *R. fruticosus*, Belluno, Italy, October, 1878, C. Spegazzini (Spegazzini, C. Supp. ad Dec. Mycol. Ital. No. 13). A. Upper surface of leaflet showing purple-bordered brown marginal discoloration on which the fungus is fruiting. ×1. B. Lower surface of same leaflet. ×1. C and D. Discolored apical region of the leaflet, showing numerous pycnidia. ×12. E. *Gloeosporium necator* Ell. and Ev. on *Rubus occidentalis*, Anna, Ill., June, 1884, F. S. Earle. Fragment of specimen in Ellis Collection New York Botanical Garden. ×3. F. *G. venetum*, section of pycnidium from C; *a*, pycnidial wall; *b*, conidia in pycnidial cavity. ×350. G. Anthracnose on stems and leaflet of wild black raspberry from a woodland, Bluemounds, Dane Co., Wis., July 26, 1938, H. C. Greene and A. E. Jenkins. ×1. Photographs by M. L. F. Foubert (A–D, and G) Lilian Guernsey (E) and Marguerite Wilcox (F).

zini's collection of *G. venetum* at Conegliano was also on *R. fruticosus*. At that place, where the youthful mycologist was living at the time (cf. 21), he collected three other fungi on that particular host. These were reported upon by his friend and teacher Prof. P. A. Saccardo (cf. 15, p. 386, p. 420, and p. 423).

Spegazzini's preparations of his *Gloeosporium venetum* evidently did not reveal to him that the more or less flattened, dark fructification visible on the upper leaf surface is actually a thin-walled pycnidium. This dark body is astomous, more or less flattened, reaching 140–185 × 48–112 μ in dimensions (Fig. 1, C, D, and F).[5] Slender unbranched hyaline sporophores, reaching 12 μ in length, are produced from practically the entire inner surface of the relatively thin pycnidial wall. Conidia in the preparations measure 4.8–5.8 × 1.6–2 μ. These somewhat smaller dimensions than in the original description could be accounted for by the shrinkage of the material in fixing.

Clearly, to avoid any further confusion, Spegazzini's pycnidial fungus on what is presumably *R. fruticosus* from the Italian Adriatic region should no longer be referred to *Gloeosporium*. Insofar as we know this is the only correct record of *G. venetum* on *Rubus*;[6] moreover, we have found no described sphaeropsidaceous fungus that seems definitely to apply to it. *Phyllosticta fuscozonata* Thuem. (2, p. 14) on leaves of European red raspberry (*Rubus idaeus* L.), also from the Adriatic region, has certain features in common with Spegazzini's *G. venetum*, such as epiphyllous, lenticular pycnidia and hyaline spores of nearly the same size. On the other hand, marginal discolorations are not described for the *Phyllosticta* leaf spot, nor is the multizonate characteristic of this leaf spot mentioned for that of the *Gloeosporium*. Unfortunately, we have no authentic material of *P. fuscozonata* for comparison with *G. venetum*. The original description of *Phyllosticta fuscozonata* is:

"*Phyllosticta fuscozonata* Thüm. nov. spec.

P[hyllosticta]. peritheciis epiphyllis, sparsis, mediis, fuscis, lenticularibus in macula magna, irregularis vel subsinuosa, sordide fusca, griseo-fusco multizonata, ferrugineo indistincte cincta; sporis cylindrico-oblongis, utrinque rotundatis, rectis, plerumque biguttulatis, hyalinis, 7–9 mm. long., 3.5–4 mm. crass.

Ad *Rubi Idaei* Lin. folia viva. Aest.—Plezzo (n. ° 778)."

Under these circumstances, we are suggesting that *Gloeosporium venetum* Speg. may be regarded tentatively as a synonym of *Phyllosticta fuscozonata* Thuem.[7]

[5] Acknowledgment is here made to Miss Marguerite Wilcox, of the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, for preparing the microscopic mounts employed in this study. These were embedded in paraffin and stained with triple stain.

[6] Briosi (3, p. 278) reported *Gloeosporium venetum* on wild rose (*Rosa*) growing at Fuipiano, Bergamo, Italy. It would not be expected that the fungus in question on rose was not pycnidial. If the specimen is still in existence it could be examined to determine whether it is not *Sphaceloma rosarum* (Pass.) Jenkins (9, p. 330).

[7] We should be glad to hear from any one who has an authentic specimen of this fungus. We have ascertained that there is none in the Mycological Collections of the Bureau of Plant Industry, the Farlow Herbarium, Harvard University, or the New York Botanical Garden.

GLOEOSPORIUM NECATOR ELLIS & EV.

In 1888, Prof. F. L. Scribner (16) reported on "Anthracnose of the raspberry and blackberry" then "wide-spread and destructive" in the United States. He noted that Prof. T. J. Burrill (5) of Illinois, had previously published an account of the disease. Scribner continued: "Professor Burrill did not name the fungus, but merely referred to it as the 'Raspberry Cane Rust,' the popular name by which it is known, stating, however, that the parasite probably belonged to the same group as the fungus that caused the disease of grapes known as Anthracnose. . . . In November 1887, Messrs. Ellis and Everhart described the fungus under consideration and, believing it to be a new species, named it *Gloeosporium necator.*"

Ellis and Everhart's (6, p. 129) description is as follows:

> "*Gloeosporium necator* E. and E.—On living canes of black and red raspberry. Sent from Evanston, Ill., by Chas. Wheeler, August, 1881, and from Cobden, Ill., by F. S. Earle, June, 1884; also received from Columbus, Mo., June 1887, from B. T. Galloway. Spots caulicolous, pale, with a slightly raised dark border, 2–3 millim. in diameter, orbicular or elliptical; spores oblong-elliptical, 5–7 × 3, oozing out in an amber-colored mass through a single opening in the center of each spot. Reported as being very injurious. *G. venetum* Sacc. (sic) [Speg.] has spores of about the same size but is a foliicolous species. The Illinois specimens were reported as *Phyllosticta necator*, but the fungus is evidently a *Gloeosporium.*"

Unfortunately Scribner (16) in a laudable attempt to avoid duplication of names accepted Spegazzini's earlier name rather than that of Ellis and Everhart. He explained that he had "no specimens for comparison, but the description given by Spegazzini of *Gloeosporium venetum* published in 1877 (sic), applies perfectly to the American fungus described by Ellis and Everhart in everything except that it is said to occur on leaves." He continued:

> "In the description of *G. necator* reference is only made to its occurrence on the canes; the fact of its being very common on the leaves was apparently unknown to the authors of the species. Recent observations have led to the discovery that no part of the plant above ground is free from the attacks of the parasite. It is occasionally seen attacking the fruit, and the petioles and veins of the leaves are often greatly disfigured by it."

Scribner's adoption of *Gloeosporium venetum* instead of *G. necator* resulted in the almost complete displacement of *G. necator*, designating a destructive pathogen on *Rubus* from America, by an unknown Italian fungus also on *Rubus*, viz. *G. venetum* Speg. An exception appears in Thaxter's (20) report of "Anthracnose of the raspberry" in Connecticut, in 1889, in which he cited the pathogen as *Gloeosporium necator* Ell. & Ev. "Numerous round or elongate white patches" are produced "on the 'canes,'" he stated, "and small yellowish spots on the leaves." These same symptoms are illustrated in figure 1, G. The small leaf spot is, of course,

entirely distinct from the continuous marginal discoloration produced by the true *G. venetum.*

Ellis evidently made no change in his description when he found that the fungus was "a *Gloeosporium.*" In the "Ellis Collection" in the New York Botanical Garden the specimens, except for the Missouri material which is lacking, still appear under the unpublished name *Phyllosticta necator.* A fragment of the specimen "on living black raspberry canes" collected at Anna (near Cobden), Ill., in June, 1884, by Earle has been made available to us for examination through the courtesy of Dr. F. J. Seaver. The numerous small cankers present on the specimen are in keeping with the technical description of them (Fig. 1, E). On their surfaces is the thick exposed stroma on which the "oblong-elliptical conidia" are borne as illustrated by Burkholder (4, fig. 16) for the conidial stage of *Elsinoë veneta.* This conidial stage is typical of the form genus *Sphaceloma* de Bary (1, also cf. 12) where Burrill indicated it should be placed. His difficulty was that he found the conidial stage from bramble so similar to *S. ampelinum* de Bary (1), type of the genus *Sphaceloma* and pathogen of grape anthracnose, that he could not satisfactorily distinguish the two. Scribner (17, p. 133) later referred to the "striking similarity" of these two fungi and published comparable drawings of acervuli (17, figs. 1511 and 1578, reproduced in 10, figs. 1, A and B). Our cultural comparison of these fungi made independently revealed their "very close resemblance in pure cultures" (Shear, 18) and the fact that they were separable when grown under parallel conditions (Jenkins, 7, 11). In cross inoculation experiments neither species affected the host of the other (11).

We are here transferring *Gloeosporium necator* to the genus *Sphaceloma* as *S. necator* (Ell. & Ev.) n. comb. The name *G. venetum* as used by American authors, of course, refers to *G. venetum* Speg.; the true *G. venetum* Speg. on *Rubus* from Italy is entirely inapplicable. The new combination naturally has no effect on the name ordinarily applied to this organism, *i.e.,* its perfect stage name *Elsinoë veneta* (Burkh.) Jenkins—Syn. *Plectodiscella veneta* Burkh. (8, p. 696).

In the absence of a specified type specimen of this fungus we suggest that Earle's specimen on black raspberry (*Rubus occidentalis* L.) be so designated. We have deposited the fragment received from Dr. Seaver in the Mycological Collections of the Bureau of Plant Industry and have indicated that it is "part of the type." The packet containing the specimen in the Ellis Collection is actually made of Professor Earle's letter transmitting the specimen to Ellis. The text of the letter written from Cobden, Ill., on June 17, 1917, reads:

"I enclose you a few young stems of black cap raspberries showing as I think the first stages of the so-called "cane rust." On a very hasty examination I find great numbers of minute simple spores. What is it? I have not been able to find any distinctive fungus in connection with these spots before."

Two days later Earle wrote Ellis again transmitting a specimen of the fungus on "Reliance" red raspberry (*Rubus idaeus*). The Wheeler specimen in the Ellis Collection bears the following label: "*Phyllosticta necator*, Evanston, Ill.?, leg. Charles Wheeler, Aug. 1881. Com. E. Williams, Montclair, N. J." These two specimens could be designated as paratype. We have no knowledge of what became of the cited specimen obtained by Galloway in Missouri. It is not available either in our Mycological Collections or in the Farlow Herbarium and as stated above has not been found in the New York Botanical Garden.

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# THE EFFECT OF LIGHT AND TEMPERATURE ON CONIDIUM PRODUCTION BY HELMINTHOSPORIUM GRAMINEUM IN CULTURE

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## INTRODUCTION

This paper presents the results of a series of trials testing the effect of light and temperature upon conidiophore and conidium production on agar media by the fungus *Helminthosporium gramineum* Rab. the causal agent of the barley stripe disease. As far as the writers are aware the only report of conidium production on agar media was that of Paxton (8) who reported conidium production on corn-meal agar from single-ascospore cultures of what he considered to be the ascigerous stage on barley straw. Negative reports on attempts to induce sporulation by this fungus in culture have been given by Dickson (3), Isenbeck (6), Ravn (9), Vogt (11) and others. Johnson (7) reported on the effects of temperature, light, moisture, aeration, hydrogen-ion concentration, various types of media, plant tissue extracts, yeast extracts, variations in nutrients, and a number of other factors upon sporulation, all his numerous trials giving negative results.

## SPORULATION ON ARTIFICIAL MEDIA

Many investigators have reported that this fungus failed to sporulate on artificial media under a variety of environmental and nutritional conditions. The success obtained by Snyder and Hansen (10) in inducing sporulation in Fusarium species by exposing cultures to diurnal variation in light suggested the use of such a method with the fungus under consideration. The influence of light upon growth and sporulation of certain fungi has been reported by Coons (2) and Harter (5) who reviewed the early literature on the subject.

In April, 1946, twenty single-spore isolations were made on potato-dextrose agar from diseased leaves from each of six lots of barley plants, each lot having been inoculated[2] during blossom in 1945 by spores from a different source of *Helminthosporium gramineum*. Ten of each of the six lots of 20 isolates growing in test tube slant cultures on potato-dextrose agar were placed in laboratory light and temperature and the other 10 of each lot were placed outdoors exposed to diurnal changes in environment with the maximum possible light without being in direct sunlight. Growth was obtained from 57 of the 60 tubes held indoors and 55 of those held outdoors. After approximately one month all tubes were examined microscopically. No

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[2] These inoculations were made by Mr. C. A. Suneson, Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Davis, California.

conidia or conidiophores were found in any of the 57 cultures held in the laboratory, while 47 out of 55 of those held outdoors had normal conidia and conidiophores. There was, however, considerable variation between isolates with regard to the number of spores produced. Many of the conidia had germinated to produce secondary conidia and mycelium, which indicated that the primary spores had been produced early in the experiment. Ten

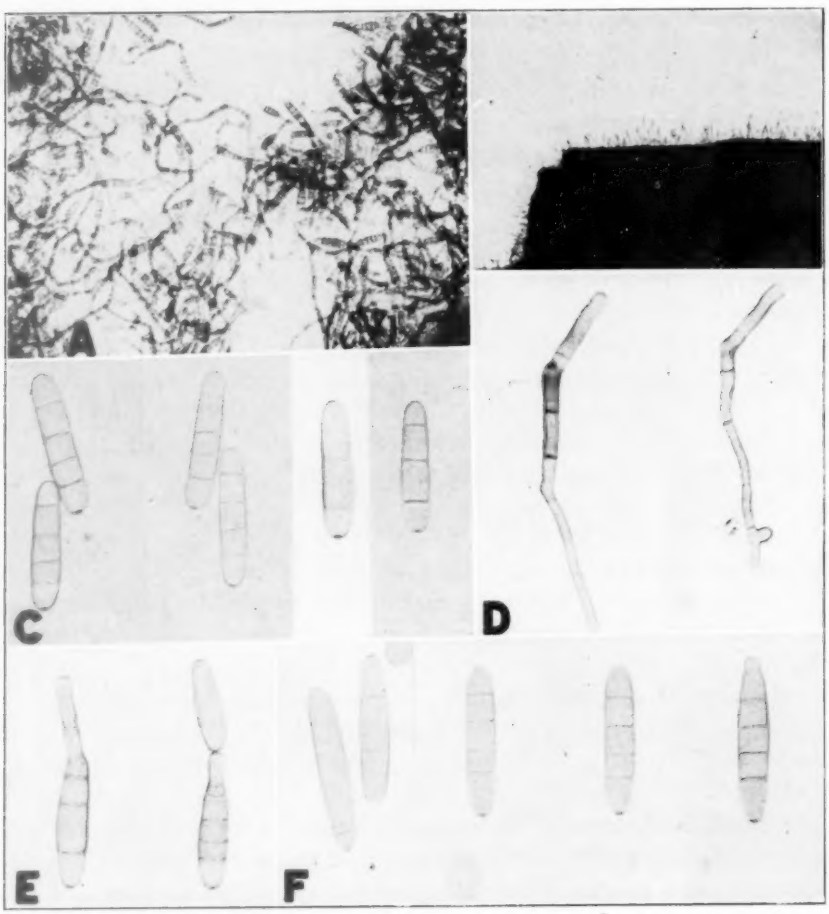


FIG. 1. Conidium production by *Helminthosporium gramineum* Rab. in outdoor environment on agar media and on diseased barley leaves. (A) Conidia and conidiophores produced on potato-dextrose agar containing sterile diseased leaf pieces (×60). (B) Section of agar block showing conidium production (×16). (C) Conidia produced on potato-dextrose agar (×255). (D) Conidiophores produced on potato-dextrose agar (×255). (E) Conidia showing formation of secondary conidia on potato-dextrose agar (×255). (F) Conidia from naturally infected barley leaves (×255).

single-spore isolations were made from an individual colony of each of the six lots of single-spore colonies, and conidia developed readily in all 60 of these under the outdoor environment within a week's time.

When those grown indoors were six weeks old the heavy aerial mycelial growth was scraped away from the surface of the agar cultures. A small

piece of the agar, 5 to 8 mm. across, containing imbedded mycelium was then transferred from each single-spore culture to potato-dextrose agar in Petri dishes and these were placed in the outdoor environment. The dishes were sealed against dust with masking tape, a method of sealing used in all subsequent tests. After three days, examination showed that 48 of the 57 pieces of inoculum were producing conidia. At the end of seven days all 57 transfers were producing abundant conidia and conidiophores of a type typical of this species (Fig. 1; B, C, D, E.).

Three isolates originally obtained by single-spore isolation from stripe diseased barley in 1944, carried by mass transfers during the two intervening years, and known to be pathogenic when tested by the method described by Arny and Shands (1), were transferred and placed in the outdoor environment. To test their relative value for conidium production, different media were used, namely, potato-dextrose agar, corn-meal agar, water agar, potato-dextrose agar and water agar to which were added several diseased leaf pieces prior to autoclaving, and potato-dextrose agar and water agar plus water extracts from diseased leaves.

Transfers of each isolate were made to test-tube slants of these media and allowed to grow outdoors for approximately three weeks before they were examined for conidia. Conidiophores and conidia were found to have developed in large numbers on those media with the highest nutritional value, namely, normal potato-dextrose agar and on this agar to which leaf pieces or leaf extracts had been added. On the less nutritive media to which leaf pieces were added the colonies developed conidia only when they grew in contact with or near such leaf pieces. The best sporulation was obtained on potato-dextrose agar to which was added the diseased-leaf pieces (Fig. 1, A). Fifty single-spore isolations were made on potato-dextrose agar from one isolate and all appeared to be similar in growth type and pigmentation. Sporulation was obtained on all of these after 1 to 2 weeks outdoors. It was noted that occasional branched conidiophores developed on the aerial mycelium of these cultures. Since little advantage was obtained from using other media, potato-dextrose agar was used in all subsequent trials.

### THE INFLUENCE OF LIGHT AND TEMPERATURE UPON SPORULATION

The effect of variations in light and temperature on several isolates known to sporulate well under outdoor environment was studied in May and June at Davis, California.[3] During this period the length of daylight was 14 to 15 hours and the average maximum and minimum outdoor temperatures were 26.8° and 8.2° C., respectively. The average maximum and minimum indoor temperatures were 25.7° and 18.0° C., respectively. Duplicate mass transfers of several nonsporulating cultures of these isolates were made to potato-dextrose agar in Petri dishes. A parallel series was carried out with pieces of nonsterilized, dried, diseased leaves placed on potato-dextrose

[3] Similar, independently conducted, unpublished studies on other fungi have recently been made by H. N. Hansen and W. C. Snyder, University of California, Berkeley, California.

agar in Petri dishes. Conidiophore and conidium production were recorded separately, because under certain conditions conidiophores were produced but few conidia developed. The results of the trial with diseased leaves were recorded under two headings according to whether sporulation occurred on the diseased leaf tissue or on the new mycelium which grew from the leaf pieces into the agar. These results are in table 1.

TABLE 1.—*Environmental factors governing sporulation of Helminthosporium gramineum*

| Environment | Nonsporulating cultures transferred to potato-dextrose agar | | Leaf pieces from diseased barley on potato-dextrose agar | | | |
|---|---|---|---|---|---|---|
| | | | Directly on diseased leaves | | On new mycelium from leaves | |
| | Conidiophore production[b] | Conidium production[b] | Conidiophore production | Conidium production | Conidiophore production | Conidium production |
| Outdoors[a] | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| Outdoors, darkened | 0 | 0 | ++++ | ++++ | 0 | 0 |
| Outdoors, daytime; Dark, 25° C., night | ++++[c] | + | ++++[c] | + | ++++[c] | + |
| Outdoors, daytime; Dark, 12° C., night | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| Dark, 25° C., 12 hr.; Dark, 12° C., 12 hr. | 0 | 0 | ++++ | ++++ | 0 | 0 |
| Inside light and temperature | 0 | 0 | ++++[c] | + | 0 | 0 |
| Artificial light, 13° C. | ++[c] | + | ++++ | ++++ | +++ | ++ |
| Artificial light, 13° C., 15 hr.; Dark, 13° C., 9 hr. | ++[c] | + | ++++ | ++++ | ++ | + |
| Dark, 8° C. | 0 | 0 | ++++ | ++++ | 0 | 0 |
| Do, 13° C. | 0 | 0 | ++++ | ++++ | 0 | 0 |
| Do, 18° C. | 0 | 0 | ++++ | ++++ | 0 | 0 |
| Do, 25° C. | 0 | 0 | ++++[c] | + | 0 | 0 |
| Do, 30° C. | 0 | 0 | +++[c] | 0 | 0 | 0 |
| Do, 35° C. | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Subject to diurnal changes in light and temperature at Davis, California, during May, 1946, but not in direct sunlight.

[b] Number of pluses indicates relative amount of conidiophore and conidium production.

[c] Extremely long conidiophores, very few bearing conidia.

On the cultures held outdoors, sporulation was abundant within 48 hours on agar, within 24 hours on leaf pieces, and within 48 hours on the new mycelium from the leaf pieces to within 5 or 6 mm. of the advancing margin. In the parallel series of cultures placed outdoors but darkened by black paper wrapping, conidiophores and conidia were not produced on the agar transfers nor on the mycelial growth from the leaves, but normal and abundant sporulation occurred directly from the mycelium in the leaf pieces. All of the cultures including the leaf pieces placed outdoors in day temperatures and light and transferred at night to a dark incubator at 25° C. produced an abundance of conidiophores 1½ to 2 times their normal length, but only 5 to 10 per cent bore conidia. However, a similar series placed

at night in a dark incubator at 12° C. produced normal and abundant conidiophores and conidia identical with those left outdoors at night.

A series held in incubators in complete darkness at 25° C. for 12 hours and then changed to 12° C. for the remaining 12 hours each day failed to produce conidiophores and conidia on the agar transfers or on the mycelium growing from the leaf pieces; however, normal sporulation occurred directly on the leaves. These results were identical with those in the series held outdoors for 24 hours each day but continuously in the dark. In a series held under laboratory light and temperatures the only sporulation obtained was directly upon the leaf pieces. Here again, as with those placed outdoors during the day and at 25° C. at night, the conidiophores were excessively long and few bore conidia. These long conidiophores with few conidia were probably the result of the higher night temperatures in the laboratory. In continuous artificial light at a constant temperature of 13° C. a moderate number of conidiophores developed on the agar cultures and mycelium from the leaves, but they were slightly longer than normal and only 10 to 15 per cent of them bore conidia. Abundant sporulation occurred directly on the diseased leaf pieces. A similar result, but with conidiophores more nearly normal in length, was obtained with the series at 13° C. with 15 hours of artificial light and 9 hours of darkness each day.

The remainder of the data presented in table 1 comprises trials in complete darkness at six constant temperatures ranging from 8° to 35° C. No conidiophores developed at any of the temperatures on the agar cultures or on the mycelial growth from the diseased leaf pieces. Abundant conidiophore and conidium development occurred on the leaves at 8°, 13°, and 18° C. The conidiophores were very short at 8°, appearing to be even shorter than the conidia. Conidiophores became progressively longer and conidia progressively shorter as the temperature increased. Secondary conidia developed abundantly at 13° and 18° C. At 25° C. conidiophore production was as abundant as that at the lower temperatures but the conidiophores ranged up to once again as long as those produced at 18° C. and only 10 to 15 per cent terminated in conidia. At 25° and 30° C. mycelial growth from the leaves was very profuse. No conidia developed at 30° C., but conidiophores were abundant and were 3 to 4 times the length of those produced at 18° C. At 35° C. there was no mycelial growth from the leaves. Abnormal, light-colored, narrow hyphae somewhat resembling conidiophores grew from the leaf but no conidia were produced.

Measurements were made of 50 conidia produced by a culture on agar medium and of conidia produced at 18° C. on diseased leaves. An attempt was made to measure only primary conidia by making the spore mounts at a time during conidium production when the primary conidia were mature and readily separated from the conidiophores and when only a few scattered secondary conidia had developed. Those from the culture had an average septa number of 4.2 and average size of 70.1 by 16.4 μ with a range in length from 48.6 to 97.6 μ. Conidia from the leaves averaged 4.4 septa and 79.3

by 14.9 μ with a range in length from 62.4 to 96.8 μ (Fig. 1, F). Thus, the two lots were very similar and compared favorably with measurements given by Drechsler (4) for this fungus.

As noted above, differences in length were observed in conidia produced from diseased leaves at different temperatures. The longer spores were produced at the lowest temperature. One-hundred conidia each were measured from lots produced at 8° and 18° C., respectively. Those produced at 8° C. had an average number of septa of 5.7 and measured 108.2 by 18.6 μ with a range in length from 82.6 to 129.0 μ. Those produced at 18° C. were considerably smaller with an average number of septa of 4.4 and dimension of 79.3 by 14.9 μ with a range in length from 62.4 to 96.8 μ.

## DISCUSSION

With the cultures used the results indicate that exposure to light, preferably daylight, is necessary for conidiophore and conidium development on agar. This was true regardless of type of nutrient within the range of those tested and found favorable for good vegetative growth. The agar cultures behaved identically with the mycelium produced from diseased leaves yet the mycelium within the diseased leaves was capable of producing conidiophores and conidia in the dark within a few hours. This indicated that the mycelium within the leaf as a result of the action of diurnal changes of outdoor environment during its development, had stored within it the necessary potentialities, whether chemical, nutritional, or otherwise, for conidium production. These potentialities were not transmitted to the mycelium growing from these leaf pieces into the agar. There was evidence indicating that a moderately high temperature during the period of light and a lower temperature during the period of darkness were conducive to best sporulation. Under these conditions sporulation on agar cultures was obtained within 48 hours. There was some evidence to indicate that an extended period of light as well as the higher temperatures employed resulted in excessively long conidiophores.

The ability to induce this fungus to sporulate in culture will make it possible to maintain single-spore isolates in a study of physiologic specialization, and to have a supply of conidia at all times for use in inoculation. It also makes it possible to positively and rapidly identify the organism when isolated in culture.

## SUMMARY

Abundant, normal sporulation was obtained within 48 hours on agar cultures of the fungus *Helminthosporium gramineum* Rab., the causal agent of the barley stripe disease, when held outdoors to expose them to diurnal changes of environment. Conidium production under these conditions was obtained on single-conidium isolations, on mass mycelial transfers, and on mycelial growth which developed from pieces of diseased leaves placed on agar.

A number of media were employed to test their relative value for sporu-

lation, but there was little evidence to indicate that any were superior to ordinary potato-dextrose agar. Various light and temperature combinations were employed in an attempt to determine the effect of each upon sporulation.

In the absence of light no sporulation was obtained on agar slant cultures or on mycelium growing from diseased leaves, either outdoors or indoors. In the presence of light but under continuous high indoor temperatures, again, no sporulation was obtained.

Light, preferably outdoor daylight, was necessary for the induction of sporulation. A few spores were produced under artificial light.

Relatively high temperatures throughout the growing period as well as extended periods of light resulted in excessively long conidiophores few of which produced conidia. This was true of agar cultures and of pieces of diseased leaves. A temperature drop during one-half of each 24-hour period gave best results. When diseased leaf pieces were placed on potato-dextrose agar and incubated in the dark at various temperatures from 8° to 35° C. to obtain sporulation it was found that conidiophore length increased and conidium length decreased as the temperatures increased.

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## PHYTOPATHOLOGICAL NOTES

*The Use of Wetting and Adhesive Agents to Increase the Effectiveness of Conidial Suspensions for Plant Inoculations.*[1]—In the course of studies on the rice "blast" disease, considerable difficulty was encountered in establishing infection and in securing a uniform distribution of leaf lesions on rice plants inoculated with aqueous suspensions of the conidia of the causal organism, *Piricularia oryzae* Cav. This was because of the high surface tension of the droplets on the waxy surfaces of the rice leaves. When the rice plants were subjected to continued wetness in the humidity tent, free moisture formed on the leaves in large droplets which, as they rolled off, removed conidia which had not yet had sufficient time to germinate and infect the plant. As a result, the leaf infections were few and generally aggregated either at the tips or at the bases of the leaves. In order to increase the effectiveness of atomizing conidial suspensions onto the plants, a series of experiments was conducted using wetting and adhesive agents, singly and in combination. The criteria used for selecting these agents were the effective reduction of surface tension, the adhesion of the solution to the plant, the non-toxicity of the agent to the conidia, and finally, the relative amount of infection resulting from the use of conidial suspensions containing the agent.

The following wetting and adhesive agents were tested by atomizing 1 per cent solutions on rice leaves: soft soap, Castile soap powder, saponin, sodium oleate, gum arabic, Micro-bentonite, Fuller's earth, calcium caseinate, gelatin, and Casco glue. In addition, single droplets of known volume were placed on rice leaves and the areas covered by the droplets were compared. By this method soft soap, Castile soap powder, and sodium oleate were selected as possible wetting agents; and calcium caseinate, Casco glue, and gelatin were selected for possible use as adhesive agents. The lowest effective concentration was then determined for each of the selected solutions and these, in turn, tested singly and in combination for relative toxicity to *Piricularia oryzae* by germinating the conidia in the respective solutions and studying their reactions. At the same time their relative effectiveness on plants was determined by taking equal volumes of suspensions containing an equal number of conidia and atomizing them onto plants. After the inoculated plants were dry they were subjected to 24 hours of continued wetness in a humidity tent to insure the establishment of infection. Further experiments were made using those agents selected from these tests. The results from the germination studies and the last two plant inoculation trials are given in table 1.

It was possible to increase the number of leaf infections per plant many times over that effected by conidia in a water suspension, and, at the same time, to obtain a more uniform distribution of leaf infections over the entire

[1] Work conducted at Camp Detrick, Frederick, Maryland, from September, 1944, to May, 1945.

TABLE 1.—*The effect of wetting and adhesive agents upon the germination of Piricularia oryzae conidia and the establishment of infection on rice plants*[a]

| Wetting and adhesive agent | Germination of conidia (per cent) | Trial A | | Trial B | |
|---|---|---|---|---|---|
| | | Total No. plants | Av. No. infections per plant | Total No. plants | Av. No. infections per plant |
| Water (Control) | 99 | 45 | 0.2 | 38 | 0.8 |
| 0.1 per cent soft soap + 0.1 per cent gelatin | 97 | 33 | 9.5 | 38 | 2.1 |
| 0.1 per cent soft soap + 0.25 per cent gelatin | 96 | ..... | ..... | 38 | 3.8 |
| 0.05 per cent Na oleate + 0.1 per cent gelatin | 90 | 44 | 5.8 | 38 | 5.3 |
| 0.05 per cent Na oleate + 0.25 per cent gelatin | 98 | 37 | 11.1 | 38 | 6.5 |
| 0.05 per cent Na oleate + 0.25 per cent Ca caseinate | 90 | 42 | 3.1 | 38 | 0.7 |

[a] Acadia rice in the 4–5 leaf stage was used.

surface of the leaf. Because of this uniformity in lesion distribution, a considerably more accurate and reliable method of determining numbers of leaf lesions was achieved, and this was used as a scoring procedure in later studies.[2] A solution containing 0.05 per cent sodium oleate and 0.25 per cent gelatin was the most effective spreader-sticker combination. All of those shown in table 1, however, were superior to the water control. Stock solutions of sodium oleate and gelatin were kept in cold storage so that conidial suspensions could be readily made when necessary. It is believed that these combinations of wetting and adhesive agents may prove useful for the application of conidial suspensions to leaf surfaces similar to those of rice.—AXEL L. ANDERSEN and B. W. HENRY.

*Systemic Infection of Downy Mildew in Soybean and Alfalfa.*[1]—In the soybean nursery at Madison, Wisconsin, in 1945 downy mildew (*Peronospora manchurica* (Naoum.) Syd.) developed about the middle of June and spread to the entire nursery before the end of the summer. In the more susceptible varieties pods at many of the nodes bore seed encrusted with oospores of the fungus as described by Johnson and Lefebvre.[2] From such seed planted in the greenhouse infected seedlings have at times been obtained with lesions apparently connected by mycelium in a systemic infection. Young plants with such systemic infection have been collected in the field by Dr. W. B. Allington and others, and in the collection by Allington characteristic mycelium of the fungus has been found in all parts of the plants through the hypocotyl and first trifoliate leaf.

[2] Andersen, A. L., B. W. Henry, and E. C. Tullis. Factors affecting infectivity, spread, and persistence of *Piricularia oryzae* Cav. Phytopath. In press.

[1] A cooperative investigation of the U. S. Department of Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, and the Wisconsin Agricultural Experiment Station.

[2] Johnson, H. W., and C. L. Lefebvre. Downy mildew on soybean seeds. U. S. Dept. Agr., Pl. Dis. Rptr. **26**: 49–50. 1942.

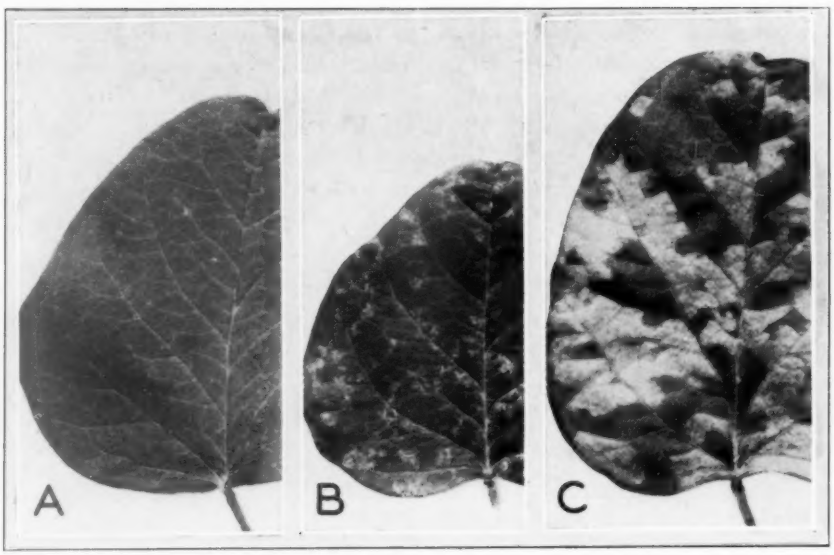


FIG. 1. Types of downy mildew lesions on soybeans. A. Noninfected leaflet; B. Leaflet of a variety having uniformly small lesions; C. Leaflet of a variety having large and often coalescent lesions.

Varieties of soybeans differed in the kind and the abundance of lesions that developed in the field during the summer; and the same difference was found when these varieties were inoculated in the greenhouse early in the following spring. In some varieties visible lesions were absent or rare in the

TABLE 1.—*Classification of soybean varieties based upon size or number of downy mildew lesions, and the percentage of encrusted seed produced in a nursery in 1945 at Madison, Wisconsin*

| Variety | Character of lesion | Encrusted seed[a] | Variety[b] | Character of lesion |
|---|---|---|---|---|
| | | *Per cent* | | |
| Richland | Large | 23.8 | Goldsoy | Large |
| Illini | do | 18.8 | | |
| Earlyana | Small | 2.3 | Flambeau | Small |
| Habaro | do | 0.5 | Kabott | do |
| Ottawa Mandarin | do | 0.3 | Pridesoy | do |
| Mandarin No. 7 | do | 0.8 | | |
| Dunfield | Rare or absent | 1.0 | Montreal Manchu | Rare or absent |
| Manchu 3 | do | 1.8 | | |
| Manchu 606 | do | 0.5 | | |
| Manchukota | do | 0.3 | | |
| Ontario | do | 0.3 | | |
| Lincoln | Large or small[c] | 7.0 | | |
| Mingo | do[c] | 3.8 | | |

[a] Based upon 400 seeds drawn from each of 4 replicates in the nursery.
[b] The five varieties listed were tested in the greenhouse only.
[c] Plants within the variety were of two kinds, one kind with large lesions, the other with small lesions.

field, and none were found in the greenhouse; in others, lesions on all plants were large (Fig. 1, C) or small (Fig. 1, B) or the variety was a mixture of plants having large or small lesions (Table 1). Varieties and selections having large lesions had outstandingly large percentages of spore-encrusted seed, reaching 41 per cent in one selection. On the other hand varieties in which lesions were rarely found still had occasional spore-encrusted seed. Whether these were borne on plants with rare or no visible lesions or came from seed mixtures remains to be determined.

Mildew infection apparently had no effect on seed yield in this nursery. A nonsignificant correlation coefficient of –0.17 (N = 67) between percentage of seeds encrusted with mildew and yield of seed in bushels per acre was obtained from a yield nursery of segregates of (Lincoln × Richland) Lincoln.

A nursery of transplanted alfalfa was also infested with mildew (*Peronospora trifoliorum* DBy.) in 1945. The nursery contained clones of unselected plants from several varieties and strains, and also closely spaced single plants obtained from selfing selected plants whose susceptibility to mildew was known. Clones and plants differed as in soybeans. Many were not infected at all. Infected plants usually had only infected leaves. A few had systemic infection to some degree, sometimes only in axillary shoots in late fall, but more often in late fall in crown shoots which were pale in color, but which survived the winter, and on which conidia were found April 13, 1946. These conidia are assumed to be the source of heavy infestation in this nursery in the following May.

The percentage of infected plants in varieties ranged from 12 per cent in Hardistan and Ladak to 60 per cent in Hardigan. Among the populations obtained by selfing susceptible plants, one of 90 plants developed in late autumn systemic infection in one or more crown shoots of each individual. The parent was infected in the same manner. Parents susceptible to leaf infection gave populations in which over half the plants were similarly infected, with a small percentage of plants in which systemic infection occurred also. Both systemic and leaf infection has been obtained from inoculation in the greenhouse; a thorough comparison of the reaction of plants in the greenhouse with that in the field has not been made. However, it appears at present that systemic infection in shoots is an important agency in the overwintering of this fungus, and that but a part of the plants that may have leaf infection are capable of this systemic invasion.—FRED R. JONES, Senior Pathologist, U. S. Department of Agriculture, and J. H. TORRIE, Associate Professor of Agronomy, Wisconsin Agricultural Experiment Station.

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Junior authorship indicated by pages in "( )"

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## ERRATA, VOLUME 35

Page 1008, line 1, *read* Pseudomonas *for* Psudomonas

Page 1009, line 39, *read* inoculation techniques *for* infection techniques

## ERRATA, VOLUME 36

Page 14, line 15, *read* Ann. Rept. 6: 84. 1892 (1893). *for* Ann. Rept. 5: 84. 1892–93

Page 22, table 1, footnote c, *read* Moderate, 5 to 10 spots per root. footnote e, *read* Weighted average of scale, none to 4, inclusive

Page 37, item 2 in literature cited, *read* Karon *for* Katon

Page 38, line 14 and Page 58, line 20, *read* verrucosans *for* verrucosa

Page 75, line 38, *read* season *for* sesaon

Page 92, line 30, *read* reduced the number of growths of Diplodia *for* reduced the Diplodia

Page 511, figure legend and Page 512, line 15, *read* abscisable *for* abscissable

Page 549, line 20, *read* and in severe form near Klamath Falls, Oregon, and Alturas, California, at elevations above 4,000 feet *for* at elevations above 4,000 feet

Page 591, line 17, *read* special media *for* media

Page 593, line 5, *read* extract as for yeast medium I *for* extract and yeast solution as for medium I

Page 611, line 16, *read* noninoculated *for* inoculated

Page 648, table 1, line 2, *read* Helipterum humboldtianum *for* Helipterum humboltianum

Page 680, line 22, *read* G. A. Gries *for* A. Gries

Page 754, paragraph 5, *read* The low humidity room was held at 75 $\pm$ 0° F. and 56–57 per cent relative humidity, and the high humidity room was held at 79 $\pm$ 3° F. and a relative humidity of 90–94 per cent

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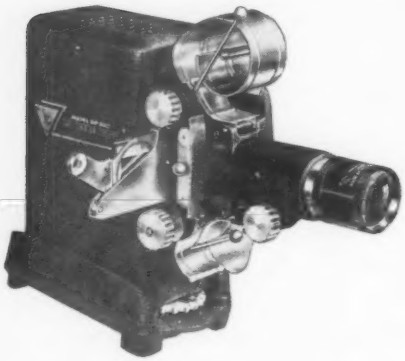

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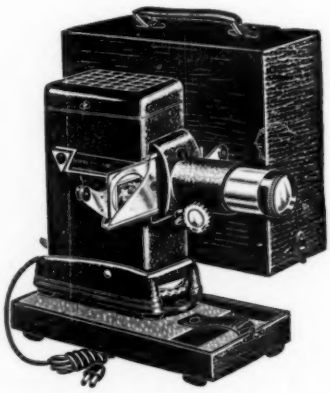

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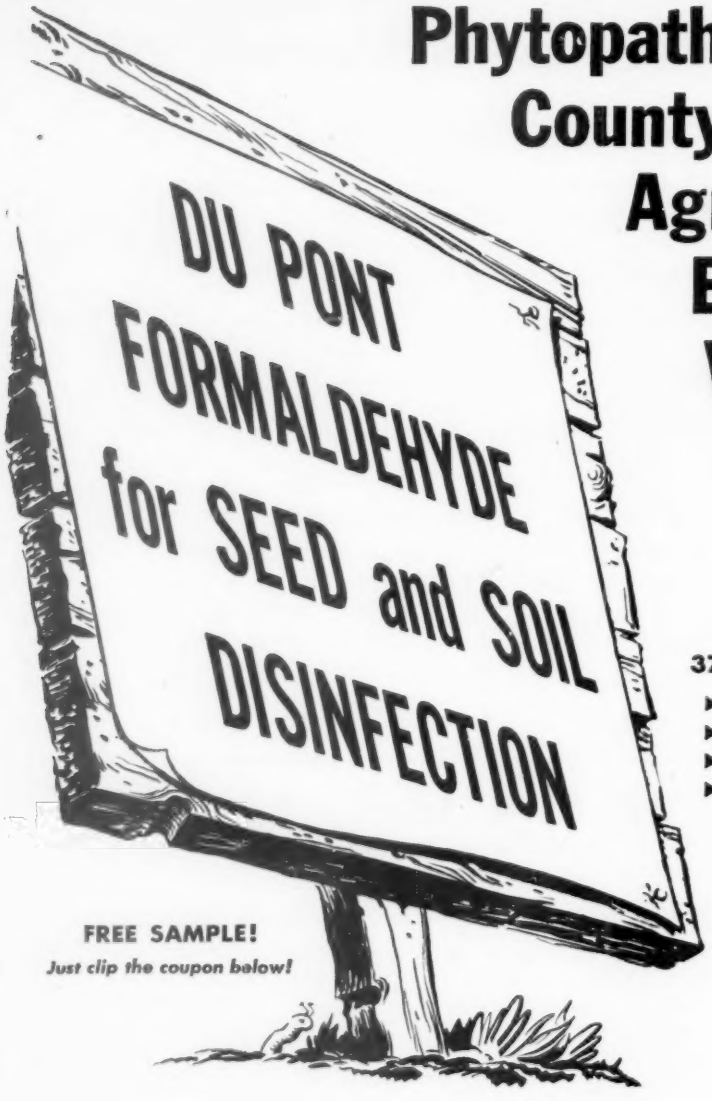


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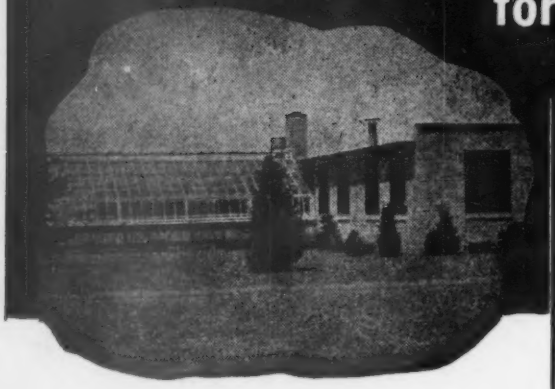

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